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SCIENTIFIC ADVISORY BOARD

MARCH 5-6, 1966

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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AGENDA

SCIENTIFIC ADVISORY BOARD MEETING

MARCH 5 - 6, 1966

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1. Administrative actions taken since September 1965

2. Consideration of applications

   a. Deferred applications

   b. Renewal applications

   c. New applications

3. Program planning:

   a. Reports of meetings, conferences and visits

   b. Plans for conferences, symposia and "round-tables"

   c. Analysis of program and discussion of areas requiring increased consideration

4. Miscellaneous

5. Future meeting dates

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

CONFIDENTIAL REPORT

SCIENTIFIC ADVISORY BOARD MEETING

Boca Raton, Flordia

December 10-11-12, 1965

Attendance

Dr. Kenneth M. Lynch, Chairman	
Dr. C. C. Little	Scientific Director, CTR
Dr. Howard B. Andervont	
Dr. Richard J. Bing	
Dr. McKeen Cattell	
Dr. Stanley P. Reimann	
Mr. T. V. Hartnett	Chairman, CTR
Mr. W. T. Hoyt	Executive Director, CTR
Mr. Simon O'Shea	Assistant to Executive Director, CTR
Dr. Robert C. Hockett	Associate Scientific Director, CTR
Dr. J. Morrison Brady	Associate Scientific Director, CTR
Dr. Vincent F. Lisanti	Scientific Associate, CTR

1. The report of actions taken at the September meeting was approved by the Board.

2. The Chairman read a letter in which Dr. Paul Kotin tendered his resignation. The Board accepted his resignation with regrets, and instructed the Executive Director to write expressing its regrets and the Board's appreciation of Dr. Kotin's services.

3. The Board discussed the recruitment of additional members. In connection therewith, the staff was authorized to invite Dr. Carl C. Seltzer of the Harvard University School of Public Health and Dr. Sheldon C. Sommers of Columbia University College of Physicians and Surgeons to the March Board meeting.

4. Dr. Bing reported to the Board on recent activities of the AMA-ERF Committee for Research on Tobacco and Health. He said the Committee had elected Dr. Chester Southam of Sloan-Kettering to its membership. He also stated that the Committee will sponsor a conference of all of its grantees to be held in Colorado Springs during the fall of 1966.

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5. The Executive Director reported on the status of the grants-in-aid program budget. During 1965, it was reported that the Board had authorized grants of $1,263,000.

He also reported that The Council has authorized a budget of $1,400,000 for 1966, which amount had been requested by the Board as its estimated requirement. It was pointed out, however, that the setting of this budget in no way implied a limitation on the availability of funds for worthwhile projects.

6. Administrative Actions

#80R10 1965 Fellowship Program. The Board authorized an additional expenditure of $1,000.00, bringing the 1965 total to $56,000.00.

#230R3 Edward E. McKee, M.D. A refund of $47.60 was received.

#290R2 William S. Murray, Sc.D. It was reported that a signed application had been received from the business officer of the grantee's institution, and the grant was activated as of September 1, 1965 in the amount of $9,500.00.

#326R1 Ira Gore, M.D. A refund of $197.79 was received.

#327R2 Philip Cooper, M.D. An additional extension from September 1, 1965 to September 1, 1966 was authorized with no additional funds.

#341R1 Thomas M. Gocke, M.D. A refund of $1,162.40 has been received. A transfer of this balance to the grantee's new institution was authorized, pending receipt of a notification of acceptance from the business officer of the State University of New York at Syracuse.

#381R2 Barbara B. Brown, Ph.D. After the staff review requested by the Board, the project renewal was activated at $15,000.00 as of July 1, 1965.

Authorization was granted to transfer grant funds in order to defray the expenses of participation for Dr. Brown and an assistant at the Latin American Congress on Pharmacology in Mexico City on October 27-30, 1965.

#397R1 Anthony P. Amarose, Ph.D. An extension from October 31, 1965 to March 1, 1966 was authorized.

#409R1 Frederik B. Bang, M.D. It was reported that Dr. Bang will be conducting a study in India during the coming year, and that his project will continue under the direction of Dr. William Hillis.

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#420 Albert B. Palmer, Ph.D. It was reported that Dr. Palmer has left the State University of South Dakota to join the faculty of the University of Toledo (Ohio). Authorization was granted to complete the project at the University of Toledo, and to transfer the remaining funds ($1,967.91) to that institution.

#477M Marcus N. Carroll Jr., Ph.D. The grantee reports that the salary of a technician is costing $2,000.00 above his budget estimate. Authorization was granted to employ such an assistant at $7,000.00. A supplementary application will be submitted to the Board in March.

7. Deferred Applications

#461M Rose Marie Pangborn $14,391.00 Approved.

#483M Herman V. Boenig, Ph.D. $33,000.00 Approved for one year.

#493M Barbara K. Watson, Ph.D. $20,702.00 Approved. The staff was instructed to transmit to her a memorandum submitted by Dr. Andervont as the opinion of the Board.

#496 Martin R. Feldman, Ph.D. $35,688.00 The Board deferred action without prejudice until its March meeting.

#498 T. C. Barnes, D.Sc. $1,150.00 The Board deferred consideration of this application pending a new application from a new institution in which the research would be conducted.

8. Renewal Applications

#80R11 1966 Fellowship Program $56,000.00 Approved.

#326R4 Ira Gore, M.D. $25,070.00 The Board approved the grant at the previous year's figure of $15,755.00, taking note that the original project was scheduled for completion at the end of 1967. A new application would have to be considered on its merits thereafter.

#335R2 William I. Fishbein, M.D. $38,500.00 The Board approved the granting of funds on a pro rata basis for the next three to six months, during which time the grantee will be encouraged to analyze current data and submit suggestions for the future conduct of the project.

#399BR2 Julia Meyer, Ph.D. $8,000.00 Denied.

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#448R1 John S. Waugh, Ph.D. $26,377.00 The Board approved the grant for a terminal year, and authorized the staff to re-negotiate the overhead category in order to determine the exact amount of the grant.

#451R1 Martin S. Protzel, D.D.S. $32,855.50 Approved.

Source: https://www.industrydocuments.ucsf.edu/docs/nwnf0000

9. New Application from Previous Grantees

#503 Richard U. Byerrum, Ph.D. $8,368.78 Denied.

#505 Robert A. Kuhn, M.D. $27,100.00 Denied.

#516 Donald M. Pace, Ph.D. $14,894.00 Approved.

#517 Albert Damon, Ph.D., M.D. $26,450.00 The Board approved the grant at an annual figure of $15,000.00.

10. New Applications

#504 Mary Bagan Dratman, M.D. $39,683.00 Denied.

#506 Roger K. Larson, M.D. $9,549.00 Approved.

#507 Budhdev Bhagat, Ph.D. $8,050.00 Denied.

#508 Z. Thaddeus Mankowski, M.D. $12,375.00 Denied.

#509 Donald J. Massaro, M.D. $17,455.00 Denied.

#510 Donald J. Massaro, M.D. $9,870.00 Approved.

#511 Leo Shatin, Ph.D. $5,583.50 Deferred. The Board referred this back to the staff. A site visit was requested for further evaluation.

#512 Albert O. Corrodi, D.O. $25,000.00 Denied.

#513 Hyman Engelberg, M.D. $28,391.00 The Board deferred action pending a site visit, which Dr. Bing volunteered to make during a forthcoming trip to California.

#514 Kurt Enslein, B.E.E. $30,530.00 The application was referred back to the staff for possible action as a special project.

#515 Seymour Werthamer, M.D. $136,016.00 The application was referred back to the staff for possible action as a special project.

#519 Louis A. Soloff, M.D. $42,500.00 The Board approved the application at an annual rate of $31,050.00

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11. The Scientific Director discussed with the Board plans for additional contract and special projects research, to be instituted at the discretion of the scientific staff. He anticipated these to be pilot studies, short-term projects and others of a character which may not suit themselves to grants-in-aid. These projects would be budgeted separately from the grants-in-aid program of the Board, and any publication resulting would bear an imprimatur distinct from that of the Scientific Advisory Board's program. He explained that the Board would be kept informed of work going forward under this program.

12. The next meeting of the Board will be held on March 5-6, 1966 in New York.

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Administrative Actions

March 5-6, 1966

ADMINISTRATIVE ACTIONS

1. The following time extensions were granted without additional funds:

#174R7 Jack P. Strong, M.D. From January 31 to July 31, 1966.
#360R2 Neal L. McNiven, Ph.D. (formerly Dorfman) From December 31, 1965 to March 31, 1966.
#415 Duane G. Wenzel, Ph.D. From January 31 to May 31, 1966.

2. The following changes in budget amounts were made:

#448R1 John S. Waugh, Ph.D. After consultation with the institutional authorities, the final figure agreed upon was $23,562.35 rather than $26,377.00, representing an adjustment in overhead from 40% to 15%.

#517 Albert Damon, Ph.D., M.D. At the December meeting the applicant had requested $26,450.00, but the Board approved only $15,000.00. The grantee was told of this decision and reported that he had re-budgeted and would need only $13,282.50. (It appears that the originally requested $26,450.00 was for two years.)

3. The following re-allocations of grant funds were authorized:

#408R1 Benjamin Burrows, M.D. Uncommitted funds in the amount of $1,500.00 for the purpose of visiting Japanese colleagues in the interests of the project.

#492 Walter M. Booker, Ph.D. Provision of tuition and fees for a graduate student.

4. The following unexpended balances were received and deposited:

#336R2 Ernest Sondheimer, Ph.D. $97.82
#354R1 Robert L. Crain, Ph.D. (formerly Katz) $21.95
#367 Herbert L. Lombard, M.D. $408.68

5. Miscellaneous

#335R1 William I. Fishbein, M.D. A balance of $6.50 remains, and it is requested that it be transferred to the new grant, #335R2.

#380R1 Daniel Cohen, D.V.M. This grant ended as of October 15, 1965, and permission was granted to use the funds remaining for purposes of the project.

#423M2 B. Eichel, D.D.S. A request for a $2,500.00 supplement to permit taking prompt advantage of a special opportunity was received on December 20, 1965. Drs. Little and Hockett approved his borrowing this amount from his grant funds, and indicated that they would recommend a supplementary appropriation in like amount at the March meeting. A memorandum covering this matter has been sent to the grant committee and is included in the book.

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#493M Barbara K. Watson, Ph.D. Through an oversight, 20% overhead was authorized. Permission is requested to allow this sum since the authorization letter has been mailed to the grantee.

The December minutes incorrectly reported the grant amount as $20,702.00 when the grant was actually authorized at $20,851.44.

#519 Louis A. Soloff, M.D. The December minutes reported the grant as $31,050.00 when the authorization letter to the grantee stipulated $31,000.00. Permission is requested to authorize the latter figure.

6. Administrative actions pending

Dr. Hans G. Keitel, Head of Pediatrics at the Jefferson College of Philadelphia, has informally requested a small grant of $500 for purposes outlined briefly in the attached letter. Dr. Little's reply is also attached.

Dr. Carl C. Seltzer has requested an appropriation of $600 for reproducing anthropometric records from the U.S. Army in order to have this material available in his own archives for correlative studies. (See attached letter.)

Dr. Freddy Homburger has asked us to provide $6,000 for a consultant fee during 1966 for Mr. Richard Walton, Consulting Engineer, who has designed the new smoking machine for certain types of mouse exposure work (inhalation studies and smoke-jet treatment). A copy of Homburger's letter (attached) describes the proposed Homburger-Walton relationship. (We must also consider whether such a fee should be paid directly to Walton or through Homburger's grant.)

atts.

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CONFIDENTIAL

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. February 18, 1966

MEMORANDUM

TO: The committee assigned to the project of Bertram Eichel, D.D.S. - No. 423M2, Dr. Little, Chm., Dr. Andervont and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Special supplement to the grant

We enclose a letter from Dr. Eichel dated December 20, 1965 in which he requested a supplementary appropriation of $2500 for use in taking advantage of a special and unforeseen opportunity which he described.

Because timing was very important, Dr. Little and I, acting as staff, authorized Dr. Eichel to "borrow" this amount from his present grant budget and undertook to recommend an equivalent supplementary appropriation at the next Board meeting, to replace the loan.

We propose to report this authorization among the "administrative actions" at the forthcoming Board meeting on March 5-6 and ask for approval of the appropriation. It seems appropriate to acquaint the project committee with these facts in advance of the meeting.

R. C. H.

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INSTITUTE OF STOMATOLOGICAL RESEARCH
SCIENCE RESOURCES FOUNDATION
57 BAY STATE ROAD
CAMBRIDGE, MASSACHUSETTS 02138
TELEPHONE 617·868·9725

December 20, 1965

Doctor Robert C. Hockett
Associate Scientific Director
The Council for Tobacco Research - U.S.A.
633 Third Avenue
New York, New York 10017

Dear Dr. Hockett:-

It was indeed a pleasure to receive Drs. Lisanti's, Little's and your memorandum of Nov. 24, 1965 re: "Impressions and Recommendations of Conference Role of Oral Cavity Research in the Study of Tobacco Use and Human Health" and to see the extent to which you relied upon and adapted my comments and recommendations into your own.

My prime purpose for writing this letter is prompted by some unexpected, yet exceedingly promising findings which have emerged from a tangential, but pertinent line of approach to our main line of attack concerning "Metabolic Interrelationships Between Tobacco Smoke and the Human Mouth", Project #Q203.

Recently, one of our colleagues developed a new gas-liquid chromatographic assay method for the detection and quantitation of carbonyls (organic ketones and aldehydes) which, to our knowledge, is unique in the sense of its greatly increased sensitivity compared to otherwise available methods and its consequent ease of application to biological systems. This assay method has been applied to the analysis of easily obtained human saliva samples of a non-smoker and heavy cigar smoker.

Time of retention curves are shown in the accompanying graph. Curve (II) gives a spectrum of compounds found in the saliva of the non-smoker harvested immediately upon arising after a full nights sleep but prior to eating, drinking, rinsing or brushing of the teeth. Over the course of the experiment some 29 unidentified components are evident suggesting at least 29 substances, most of which undoubtedly have not been described in human saliva before, and some of which may not have been described in human fluids or, for that matter, biological samples of any kind before.

Curve (III) gives a spectrum of compounds for a comparable sample of saliva obtained from the same non-smoker the following morning immediately upon awakening, but after smoking (without inhaling) 4 cigarettes

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwn0000

(Winston). It is evident from the retention time 0 to 10 minutes that a great deal of one, two or three carbon carbonyl compounds are trapped in the saliva, component 1. In addition, three new components - 2, 3 and 4, not present in the saliva without smoking, also are trapped and appear in the saliva after smoking. From their retention times, 27.2 minutes, 33.6 minutes, and 40.1 minutes, these components are either lower molecular weight hydroxy carbonyls or carbonyl compounds of eleven or more carbon atoms. The many components (5 to 17) in the non-smoker's saliva sample without or after smoking, retention time 6.1 minutes to 31.0 minutes, represent a possible host of three to eleven carbon carbonyl compounds apparently unrelated to tobacco smoke. In addition two very long retention time carbonyls, about 50.0 minutes and 57.0 minutes, also are normally present in saliva, components 18 and 19, respectively.

Curve (I) shows the spectrum of compounds found in a comparable saliva collected from an habitual cigar smoker upon awakening in the morning after a full night's sleep, but before eating, drinking, rinsing, brushing of teeth or smoking. Much of the detail, over the range of 6 to 31 minutes found in the saliva of the non-smoker, appears to be absent in that of the habitual smoker. The particularly large peaks, possibly components 18 and 19, may be the result of using a still newer isolation technique designed to recover components of low volatility in larger quantity. It is anticipated that future collections of saliva from the non-smoker might show intensification of peaks. In addition, although the analytical method employed here has been established as quantitative, optimum column conditions must be realized for separation of these components. Such improvement would naturally result in precise retention times and lead to identification of the components.

These results and emerging perspective suggest that bio=assays or tests relating to the tobacco smoking habit may become a reality. In addition, identification of these compounds could lead to definitive experiments aimed at establishing essential metabolic interrelationships between tobacco, tobacco smoke products and the human oral cavity or other biological systems of concern.

Several practical factors, mostly economic however, are operating which work against this probable breakthrough becoming an effective one. The equipment which is required for this new and novel situation is quite expensive. Before large sums of money are requested, we believe it would be most wise to pursue a feasibility study to verify and extend the initial findings. Thus far we have been using and are continuing to use part of the funds granted under Project #Q203 to defray current costs. The advantage here is that we have use of the necessary equipment at the present time on an interim basis at low cost. Unfortunately this advance expenditure of funds is a luxury that the limited budget available under Q203 can ill afford.

Consequently, I am taking the liberty to make this informal report a letter of request for the sum of two thousand and five hundred dollars ($2,500.00) to complete a quick feasibility study along these lines within the next 45 to 50 days. Then if the results continue on their promising way and provide ample

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justification, we will submit a formal proposal for the March 1966 meeting of the CTR - U.S.A. to cover equipping the necessary laboratory, salaries etc. for the full development, exploitation and program integration of this approach.

In the meantime our metabolic studies under Q203 are in progress.

Wishing you, Drs. Little and Brady a very Merry Christmas and most Happy New Year, I remain,

Sincerely yours,

Bertram Eichel

Bertram Eichel, D.D.S.
Director

BE/md

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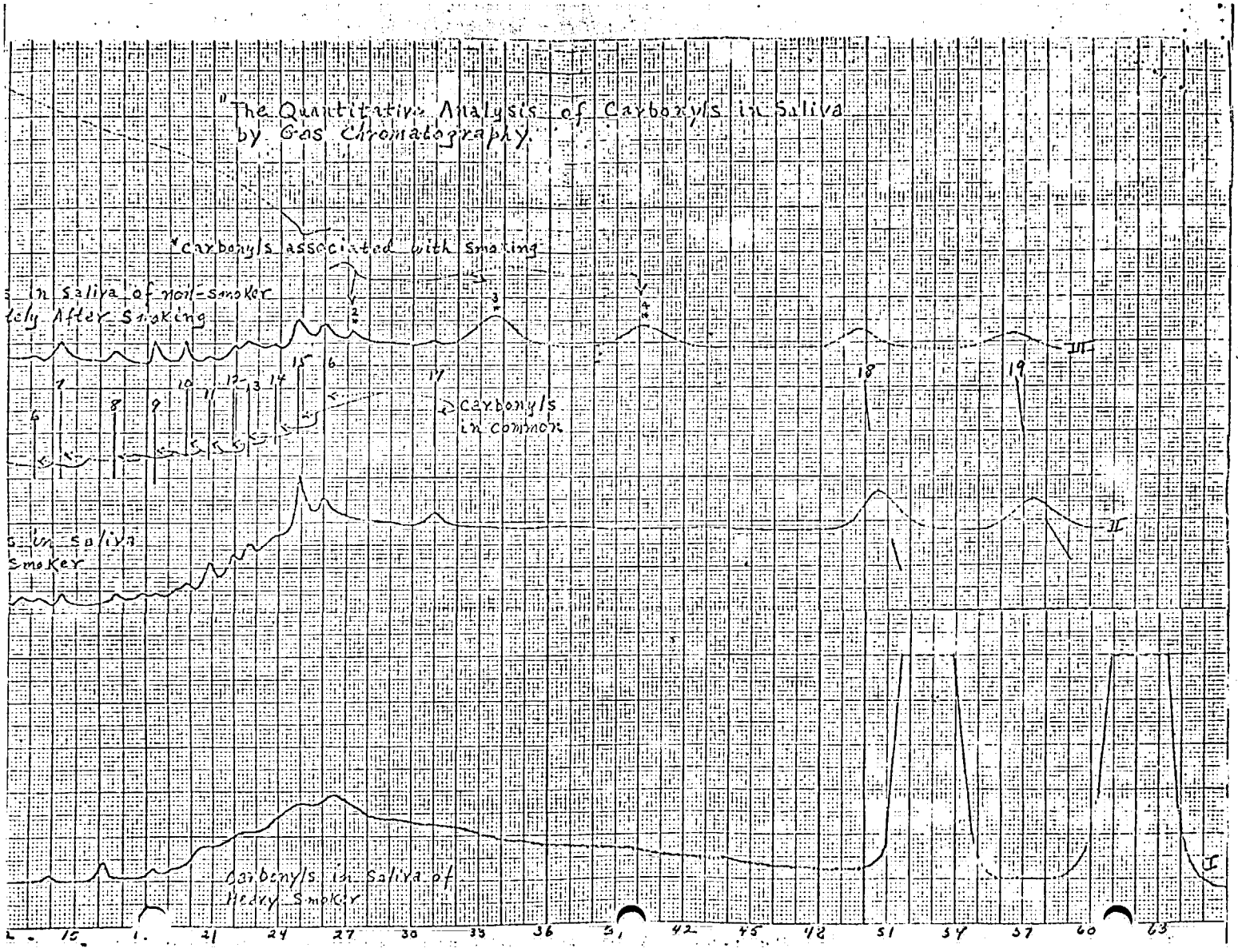
Source: https://www.industrydocuments.ucsf.edu/docs/nwnd0000


"The Quantitative Analysis of Carbonyls in Saliva by Gas Chromatography.
Carbonyls associated with Smoking
in saliva of non-smoker
tely After Smoking
Carbonyls in common
in saliva
Smoker
Carbonyls in Saliva of Heavy Smoker
I
II
III
15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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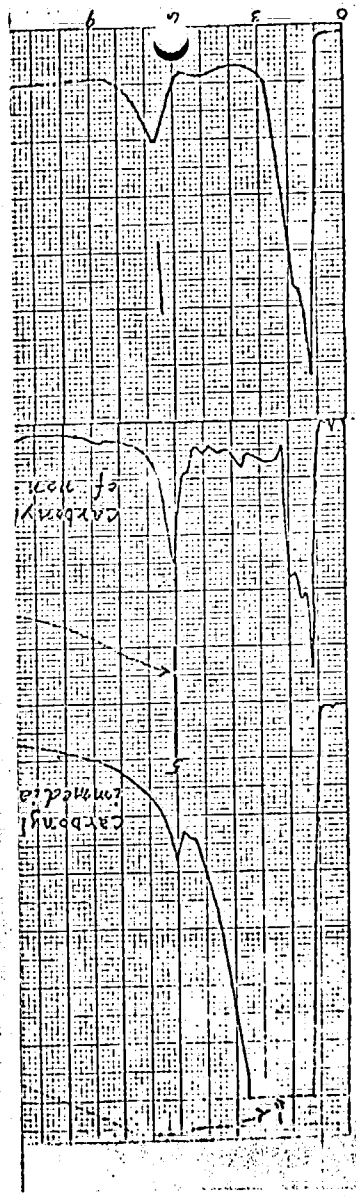

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE JEFFERSON MEDICAL COLLEGE OF PHILADELPHIA
1025 WALNUT STREET
215 WA 3-1100

HANS GEORGE KEITEL, M.D.
PROFESSOR AND HEAD OF THE
DEPARTMENT OF PEDIATRICS

January 3, 1966

Dr. R. C. Hockett
Council Tobacco Research
633 3rd Ave.
New York, N. Y.

Dear Dr. Hockett:

We have obtained evidence that a higher percentage of new born infants whose mothers do not smoke weigh over 140 ounces at birth than infants whose mothers smoke.* It is known that perinatal morbidity is higher in infants with a birth weight over 140 ounces.

We would like a small grant (of $500.00) to obtain additional information including the gestational history; this would enable us to complete the study in a far more meaningful way. Incidentally, the incidence of prematurity was the same for the smoking and non-smoking mother.

Yours truly,

Hans G. Keitel, M. D.

HGK/rg

* Difference is significant, statistically.

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RCH

February 4, 1966

Hans G. Keitel, M.D.
Professor and Head
Department of Pediatrics
The Jefferson Medical College
of Philadelphia
1025 Walnut Street
Philadelphia, Pennsylvania

Dear Doctor Keitel:

Since Doctor Hockett is away on a somewhat lengthy trip, your letter of January 3rd has been referred to me.

We have supported certain work in the field of smoking, gestation and birth weight and are also interested in such studies under other auspices.

In order to form a basis for comparison of your proposed study with this other work, it would help us to have certain additional information as to the makeup of the population which you plan to study including numbers of individuals, age distribution, economic status and method of collecting data (i.e., prospective or retrospective).

If you could provide such a more detailed statement it will be of great help to us.

Sincerely yours,

Clarence C. Little
Scientific Director

CCL:ek

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Source: https://www.industrydocuments.ucsf.edu/docs/dlwn0000

HARVARD UNIVERSITY
SCHOOL OF PUBLIC HEALTH

DEPARTMENT OF NUTRITION
CABLE ADDRESS: NUTHARV

665 HUNTINGTON AVENUE
BOSTON, MASSACHUSETTS 02115

February 18, 1966

Dr. Robert C. Hockett
Council for Tobacco Research, U. S. A.
633 Third Avenue
New York, New York 10007

Dear Bob:

Persuant to our telephone conversations relative to your offer of financial assistance in retrieving from the U. S. Army the anthropometric records of approximately 100,000 veterans of World War II, the following information gives costs needed for the present retrieval operation:

Reporduction of 100,000 cards at $3.10 per thousand	$310.00
Duplication 39 rolls microfilm at $4.50 per roll	175.00

This amounts to approximately $485 and there will be additional costs for transportation and clerical work necessitating approximately $600.

Dr. Damon and I are naturally delighted that the Council for Tobacco Research will cover this expense for us. I suggest that in order to avoid complications of giving this money to the University and then having additional overhead added that it may be desirable to have a check sent to me personally and I will submit to you the bills covering the expenditures. However, if you want to do this through the University, this can, of course, be arranged.

With many thanks and looking forward to our meeting on March 5, I remain

Sincerely yours,

Carl C. Seltzer, Ph.D.

CCS:jr

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BIO-RESEARCH CONSULTANTS, INC.

January 18th
1966

Mr. Richard Walton
10 West Hill Place
Boston, Massachusetts

Dear Dick:

This is to confirm our conversation of January 17th which we held to decide the extent of your compensation for consultant work on our tobacco research project and to agree on the cost of the machines which we have obtained and which we will obtain in the future from you.

It is my understanding that the Tobacco Research Council reimbursed you for your travel expenses in 1964-1965 and paid you a consultant's fee of $2,000 covering your work through December 31, 1965.

We have obtained one prototype smoking machine from you and will obtain another one shortly. We shall pay you $2,000 for the manufacture of each of these machines, or a total of $4,000.

I have been told that the Tobacco Research Council will consider to add to our budget the sum of $6,000 for your consultant fee for 1966.

We need your continued assistance to modify and further develop the present prototype smoking machine and to invent and construct a prototype of a machine for the automated preparation of smoke condensates. We shall purchase from you this prototype when ready and possibly additional machines of the exposure or condensate type, and we anticipate an additional purchase of no less than $4,000 during 1966.

In the event that the condensate machine requires special gears or other machine parts out of the ordinary and costing more than $250. each, we shall purchase such parts separately. It is, however, understood that you will provide the ordinary parts costing each less than $250.

I hope that this reflects correctly our discussion and satisfies you in every respect.

Sincerely yours,

FH:bhn

Freddy Homburger, M.D.

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List of Applications

APPLICATIONS TO BE CONSIDERED

Grant #	Principal Investigator	Amount Requested	Anticipated Duration	Last Grant	Category	Subcommittee
Applications deferred from previous meeting						
513	Engelberg	28,391.00	3 yrs.		Cardiovascular	Cattell, Little Jacobson
Renewal applications						
24R10	Larson	25,565.00	1 yr.	25,189.00	Miscellaneous	Cattell, Little Lynch
307R5	Bellet	22,102.40	1 yr.	16,212.00NC	Cardiovascular	Cattell, Bing Jacobson
310R5	Bing	11,671.00	1 yr.	10,995.00	Cardiovascular	Cattell, Lynch Jacobson
362R3	Manos	12,040.00	1 yr.	15,340.00 T	Virus	Jacobson, Lynch Little
405R2	Segal	55,191.20 T	3-5 yrs.	45,940.00	Pathol-Diagnostic	Reimann, Lynch Jacobson
415R1	Wenzel	11,882.00	1 yr.	9,685.00NC	Cardiovascular	Cattell, Bing Little
436R2	Aviado	19,250.00 T	1 yr.	13,418.00	Physiology	Bing, Cattell Jacobson
447R1	Schaeppi	21,400.00 T	2 yrs.	9,775.00	Pharmacology	Bing, Jacobson Cattell
466R1	Hoff	15,000.00	3 yrs.	15,000.00	Pharmacology	Cattell, Bing Jacobson
491R1	Long	7,642.00	1 yr.	8,220.00	Pharmacology	Cattell, Bing Jacobson
492R1	Booker	17,205.00	2 yrs.	17,205.00	Pharmacology	Cattell, Bing Jacobson
New applications from former grantees						
527	Heimstra	9,487.00	18 mos.	6,722.00	Psychosomatic	Cattell, Little (Seltzer)
528	Buckingham	31,624.00	3-5 yrs.	35,436.00	Physiology	Jacobson, Bing Reimann
530	Bellet	33,206.31	3 yrs.	16,212.00	Cardiovascular	Cattell, Bing Jacobson
531	Burrows	10,350.00	2 yrs.	9,270.00	Physiology	Jacobson, Bing Lynch
532	Manos	9,900.00	3 yrs.		Virus	Jacobson, Lynch Little
533	Furst	19,663.00	1 yr.	15,295.00	Bioassay	Jacobson, Andervont, Reimann Little
536	Haddy	27,340.00	1-2 yrs.	27,340.00	Cardiovascular	Bing, Cattell Jacobson

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APPLICATIONS TO BE CONSIDERED

Grant #	Principal Investigator	Amount Requested	Anticipated Duration	Last Grant	Category	Subcommittee
New Applications						
518	Weiss	3,220.00	1 yr.		Biossay	Andervont, Littl Cattell
521	Biancifiori	11,100.00	3 yrs.		Carcinogenesis	Andervont, Littl Jacobson
522	Bicks	81,200.00	2 yrs.		Physiology	Jacobson, Lynch Reimann
523	Jones	35,600.00	4 yrs.		Virus	Jacobson, Bing Lynch
524	Quigley	7,392.00	1 yr.		Oral Cavity	Lynch, Reimann Little
525	Quigley	4,314.00	1 yr.		Oral Cavity	Lynch, Reimann Little
526	Larson	17,424.00	2 yrs.		Chronic Pulmonary Diseases	Jacobson, Reiman Little
529	Manhold	7,430.15	18 mos.		Oral Pathology	Reimann, Andervont, Little
534	Goldhaber	24,414.00	5 yrs.		Chemistry	Reimann, Andervont, Little
535	Thomas	6,800.00	1 yr.		Bioassay	Little, Andervon Reimann

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CURRENT GRANTS

(By Subject)

BIOASSAY, CARCINOGENESIS & TISSUE CULTURE

		Activated	Amount	Percent of Total Awards
Bioassay				
338	F. Homburger	9/1/65	59,096	
340	C. H. Steffee	6/1/65	17,576	
456	F. Homburger	1/1/66	70,000	
			146,672	11%
Carcinogenesis				
25	R. W. Weller (Hawthorne)	5/1/65	1,050	
276	K. M. Lynch & F. A. McIver	6/1/65	29,542	
278	R. C. Reynolds (Montgomery)	9/1/65	21,863	
413	C. Leuchtenberger	10/1/65	30,955	
451	M. S. Protzel	2/1/66	32,855	
483	H. V. Boenig	1/15/66	33,000	
485	S. L. Kauffman	9/1/65	17,940	
			167,205	13%
Tissue Culture				
361	G. O. Gey	9/1/65	43,435	
373	D. M. Pace	4/1/65	14,078	
516	D. M. Pace	2/1/66	14,894	
			72,407	6%
			$386,284	30%

1005101155

Source: https://www.industrydocuments.ucsf.edu/docs/glxw0040

CARDIOVASCULAR, PHARMACOLOGY & CHEMISTRY

		Activated	Amount	Percent of Total Awards
Cardiovascular				
307	S. Bellet	7/1/65	16,212	
310	R. J. Bing	7/1/65	10,995	
320	C. E. Cross (Salisbury)	7/1/65	14,350	
326	I. Gore	1/1/66	15,755	
344	W. Redisch	7/1/65	12,379	
398	K. M. Moser	10/15/65	12,564	
402	H. D. McIntosh	10/1/65	13,316	
415	D. G. Wenzel	2/1/65	9,685	
433	F. J. Haddy	7/1/65	23,270	
467	T. C. Westfall	9/1/65	8,000	
472	J. E. P. Toman	4/1/65	15,275	
477	M. N. Carroll Jr.	7/1/65	13,430	
489	E. T. Angelakos	8/1/65	16,120	
491	J. P. Long	6/1/65	8,220	
			189,571	15%
Pharmacology				
206	E. F. Domino	1/1/66	32,303	
381	B. Brown	7/1/65	15,000	
447	U. Schaeppi	6/1/65	9,775	
466	E. C. Hoff	6/1/65	15,000	
492	W. M. Booker	7/1/65	17,205	
			89,283	7%
Chemistry				
223	H. S. McKennis Jr.	7/1/65	52,598	
351	S. H. Wender	8/1/65	28,980	
448	J. S. Waugh	2/1/65	23,562	
486	A. A. Albanese	9/1/65	13,869	
			119,009	9%
			$397,863	31%

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwa0000

EPIDEMIOLOGY, PSYCHOSOMATIC & STATISTICS

		Activated	Amount	Percent of Total Awards
Epidemiology				
335	W. I. Fishbein (6 mos.)	1/1/66	19,250	
380	D. Cohen (6 mos.)	4/15/65	7,178	
455	C. L. Rose	4/1/65	10,000	
471	H. I. Russek	8/1/65	17,000	
487	H. L. Lombard (15 mos.)	7/1/65	21,160	
510	D. J. Massaro	2/1/66	9,870	
			84,458	7%
Psychosomatic				
384	C. B. Thomas	9/1/65	12,000	
390	P. H. Knapp	10/1/65	8,598	
441	G. M. Smith	7/1/65	9,274	
517	A. Damon	2/1/66	15,000	
			44,872	3%
			$129,330	10%
LUNG PHYSIOLOGY				
385	S. Buckingham	7/1/65	35,436	
408	B. Burrows	7/1/65	9,270	
436	D. M. Aviado	4/1/65	13,418	
478	S. Ayres	7/1/65	22,890	
479	A. Spock	7/1/65	15,487	
497	A. A. Liebow	10/15/65	8,890	
519	L. A. Soloff	2/1/66	31,000	
			$136,391	11%
PATHOLOGICAL DIAGNOSTIC				
405	M. S. Segal	7/1/65	45,940	
423	B. Eichel	8/1/65	17,950	
430	B. J. Miller	7/1/65	6,035	
			$69,925	5%

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Source: https://www.industrydocuments.ucsf.edu/docs/plwn0000

VIRUS, PREVIOUS INFECTIONS

		Activated	Amount	Percent of Total Awards
Virus				
290	W. S. Murray	9/1/66	9,500	
362	J. P. Manos	5/1/65	15,340	
409	F. B. Bang	10/1/65	20,946	
493	B. K. Watson	4/1/66	20,851	
506	R. K. Larson	1/1/66	9,549	
			$76,186	6%
ORAL CAVITY				
461	R. M. Pangborn	7/1/66	$14,391	1%
MISCELLANEOUS				
24	P. S. Larson	7/1/65	25,189	
80	Fellowship Program	6/1/66	56,000	
			$81,189	6%
			$1,291,559	100%

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CURRENT GRANTS

(By Date of Activation)

No.	Grantee	Date	1965	1966
206	E. F. Domino	1/1		32,303
326	I. Gore	"		15,755 NC
335	W. I. Fishbein (6 mos.)	"		19,250 NC
456	F. Homburger	"		70,000
506	R. K. Larson	"		9,549
483	H. V. Boenig	1/15		33,000
415	D. G. Wenzel	2/1	9,685 NC	
448	J. S. Waugh	"		23,562 T
451	M. S. Protzel	"		32,855
510	D. J. Massaro	"		9,870
516	D. M. Pace	"		14,894
517	A. Damon	"		15,000
519	L. A. Soloff	"		31,000
			9,685	307,038

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Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

No.	Grantee	Date	1965	1966
373	D. M. Pace	4/1	14,078 NC	
436	D. M. Aviado	"	13,418	
455	C. L. Rose	"	10,000	
472	J. E. P. Toman	"	15,275	
493	B. K. Watson	"		20,851
380	D. Cohen (6 mos.)	4/15	7,178	
25	R. W. Weller (Hawthorne)	5/1	1,050	
362	J. P. Manos	"	15,340 T	
80	Fellowship Program	6/1		56,000
276	K. M. Lynch & F. A. McIver	"	29,542 T	
340	C. H. Steffee	"	17,576 NC	
447	U. Schaeppi	"	9,775	
466	E. C. Hoff	"	15,000	
491	J. P. Long	"	8,220	
			156,452	76,851

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwx0000

No.	Grantee	Date	1965	1966
24	P. S. Larson	7/1	25,189	
223	H. S. McKennis Jr.	"	52,598 NC	
307	S. Bellet	"	16,212 NC	
310	R. J. Bing	"	10,995	
320	C. E. Cross (Salisbury)	"	14,350 T	
344	W. Redisch	"	12,379 T	
381	B. Brown	"	15,000 T	
385	S. Buckingham	"	35,436 NC	
405	M. S. Segal	"	45,940	
408	B. Burrows	"	9,270 T	
430	B. J. Miller	"	6,035 T	
433	F. J. Haddy	"	23,270 T	
441	G. M. Smith	"	9,274 T	
461	R. M. Pangborn	"		14,391
477	M. N. Carroll Jr.	"	13,430	
478	S. Ayres	"	22,890 NC	
479	A. Spock	"	15,487 NC	
487	H. L. Lombard (15 mos.)	"	21,160	
492	W. M. Booker	"	17,205	
351	S. H. Wender	8/1	28,980 NC	
423	B. Eichel	"	17,950	
471	H. I. Russek	"	17,000 NC	
489	E. T. Angelakos	"	16,120	

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Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

No.	Grantee	Date	1965	1966
278	R. C. Reynolds (Montgomery)	9/1	21,863 T	
290	W. S. Murray	"	9,500 T	
338	F. Homburger	"	59,096	
361	G. O. Gey	"	43,435	
384	C. B. Thomas	"	12,000	
467	T. C. Westfall	"	8,000	
485	S. L. Kauffman	"	17,940 NC	
486	A. A. Albanese	"	13,869 NC	
			631,873	14,391

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

No.	Grantee	Date	1964	1965
390	P. H. Knapp	10/1		8,598
402	H. D. McIntosh	"		13,316 NC
409	F. B. Bang	"		20,946
413	C. Leuchtenberger	"		30,955
398	K. M. Moser	10/15		12,564 T
497	A. A. Liebow	"		8,890
				95,269

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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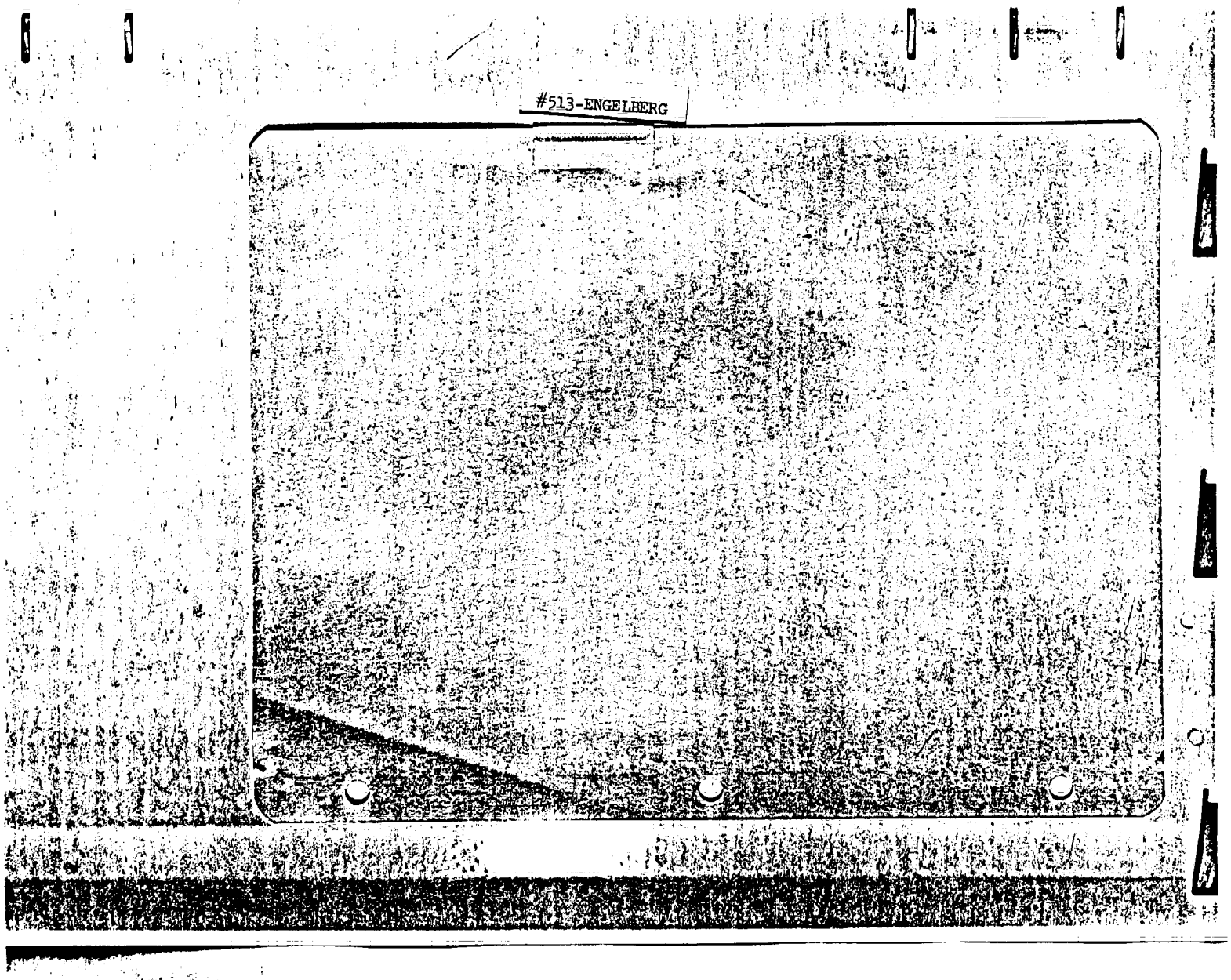

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CONFIDENTIAL

THE COUNCIL FOR TOBACCO RESEARCH - U. S. A.

February 18, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Site visit to Dr. Hyman Engelberg of Cedars-Sinai Medical Center, Los Angeles, California. No. 513.

On February 6, I visited Dr. Hyman Engelberg in his Los Angeles laboratory to discuss further the application (#513). This was discussed at the December meeting of the Board (and should be in your files), but action was deferred and a site visit prescribed. It was hoped that Dr. Bing could pay the visit but this turned out to be impossible to arrange before the March 5-6 Board meeting. However he kindly furnished me the following comment from a colleague in the field of hematology:

> "This is an interesting protocol. I am, however, wondering as to why the investigator is not planning to perform other coagulation tests in order to clarify the possible factor affecting the thrombus formation time. A complete coagulation studies to include recalcification time, partial thromboplastin time, prothrombin time, thrombin time, Factor V, fibrinogen, antithrombin, euglobulin lysis time, profibrinolysin, antifibrinolysin and platelet counts may throw further light on this problem. It seems to me that in conjunction with the proposed tests, a complete coagulation profile may be very useful. I am not aware of any report describing the effect of smoking on various coagulation tests. His approach otherwise looks very reasonable and worthwhile".

This comment was shown to Dr. Engelberg without revealing its origin. His reply was that all these things obviously ought to be done sooner or later. He does not want to add them to his program at the present moment because he does not have a skilled technician for such measurements available at the moment. He would rather spend his own time for the present on various extensions of this particular measurement that he has developed. The features that recommend it to him are its speed, simplicity and reproducibility. He feels that many of the other tests are tricky and poorly reproducibile, several having their own advocates with resultant controversy and confusion. He says he would gladly cooperate with any hematologist whom we might wish to designate and would not only furnish duplicates of his instrument (it is relatively inexpensive) but train technicians in its use. He would be glad to have cooperation on study of the relations between these tests and his own. He believes that the early change he observes is antecedent to the coagulation process.

He showed me his instrument, of which he has several duplicates, and I was impressed by the evidence of the replicability of results. Timing is essential and the whole test must be carried out immediately, with timing, after drawing the blood sample. Hence the need for several instruments.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH - U. S. A.

February 18, 1966

MEMORANDUM - Cont'd.

Both epinephrine and nor-epinephrine produce a shortening of the "thrombus formation time" (see attached charts). Changes in free fatty acids in the serum seem to bear a kind of reciprocal relation to the "time of thrombus formation", but these changes are prevented by previous administration of a big dose of nicotinic acid. Hence he thinks that free fatty acids are not mediators of the effect.

I gave him some nicotine bitartrate in ampoules several weeks ago and he has made a few observations on the effects of intravenously administered nicotine on the "thrombus formation speed". As shown on another chart the effect of nicotine is similar to the effect of epinephrine or nor-epinephrine. This suggests that the nicotine effect might be mediated through the catecholamines. This will be studied by use of agents that should block action of the latter to see whether the nicotine effect persists or not.

Engelberg is also comparing the effects of catecholamines and nicotine administered to the subject before drawing blood and added to the blood after drawing. Effects are not identical, which suggests that they are mediated through some chain of events in the body rather than the result of direct action.

He has already shown that adenosine diphosphate has a direct effect on this measurement when added in vitro and that there are variations in the levels of this agent in freshly drawn blood. Also adenosine diphosphate tends to increase with time in drawn blood in vitro.

On the whole Dr. Engelberg pretty well convinced me that he has enough to do to keep quite busy for awhile without undertaking a lot of "conventional" hematological tests in parallel.

This does leave the problem of "bridging" between this test and other kinds of observations in order to get some idea of the clinical meaning of a "shortened thrombus formation time".

Because of its speed, simplicity and replicability the test could be included in prospective studies of large populations, such as the Framingham study. I raised this suggestion and Engelberg liked it. The Framingham group includes individuals who have developed clinical cardiovascular disease and many others who have not. A large body of data has been collected and recorded on all and is being kept up to date by re-examinations. We now have a finger in the project through Damon, Seltzer and McNiven and could perhaps interest them in including this test in their data collection. If so, Engelberg is ready to cooperate. Of course, we could encounter an impasse, since they might want evidence of the significance of the test before undertaking to include it!

Engelberg is, of course, a clinically oriented man in private practise. He impressed me, however, as being alert, energetic, a clear thinker and rather well-read. It might be well worth while to give him his head for a year and see what results. The study has a possibility of direct bearing on our main targets. If the Framingham idea could be worked out and/or cooperation of a hematologist arranged for an integrated study, the values should be enhanced.

R.C.H.

Personal Opinion:

The Engelberg proposal will be placed on the agenda for March 5-6 as a deferred application.

P.S. : A manuscript just received from Dr. Engelberg is enclosed: The Effect of Catecholamines Upon the In Vitro Thrombosis of Human Blood. Engelberg and Futterman.

R.C.H.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#513

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Application For Research Grant

Date: October 22, 1965

1. Name of Investigator: HYMAN ENGELBERG, M.D.

2. Title: Attending Physician
Cedars of Lebanon Hospital

3. Institution & Address: Cedars-Sinai Medical Center
Cedars of Lebanon Hospital Division
4833 Fountain Avenue
Los Angeles, California

4. Project or Subject: Smoking and the Thrombosis of Human Blood
Mechanism and Prevention

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

This research is based upon the use of human blood and the Chandler technique which produces a true platelet-head thrombus in vitro rather than a clot. This distinction is an important one (Poole, J.C.F. and French, J.E., J. Atherosclerosis Research 1:251, 1961) since factors involved in clotting may or may not have the same relevance to thrombosis. The proposed research is also based upon the results of a pilot study I have completed in 60 subjects which indicates that there is a statistically significant increased thrombotic tendency, demonstrable in vitro, shortly after smoking. (J.A.M.A. 193:1033, 1965-Sept. 20) (Reprint enclosed).

The mechanisms underlying the markedly increased incidence of myocardial infarction in cigarette smokers have been the subject of much investigation. Nicotine releases epinephrine with a variety of resultant cardiovascular effects which have not been definitely related to the production of atherosclerosis or thrombosis (Blackburn, H., Brozek, J., Taylor, H.L. and Keys, A.: Ann. N.Y. Acad. Sc. 90:277-289, 1960). In a pilot study of 60 smokers (abstract enclosed) I have found that the thrombosis time after smoking was shortened in 34 individuals. The change was highly significant being over 6 times the standard deviation of the method (0.45 minutes as determined by 10 duplicate tests). The effect of smoking on the clotting time was much less than the effect upon the thrombosis time. These preliminary results indicate that there is an accelerated thrombotic tendency soon after smoking in many cigarette smokers. In the presence of coronary atherosclerosis this

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

may well be a major additional factor precipitating an acute coronary thrombosis. Heretofore, discussions of smoking in relation to heart disease have involved the effects of smoking on the work of the heart or on coronary flow with little or no mention of coagulation or thrombotic factors (Symposium on the Cardiovascular Effects of Nicotine and Smoking, Ann. N.Y. Acad. Sc. 90:5-344, 1960, and Roth, G.M. & Schick, R. M.. Effect of Smoking on the Cardiovascular System of Man, Circulation 17:443-459, 1958).

In 1958 a relatively simple method was developed for the in vitro production of thrombi using recalcified citrated whole blood (Chandler, A. B. Lab Investigation 7:110, 1958). Essentially the method involves placing recalcified blood in a plastic tube which is then formed into a closed loop and rotated at a speed approximating that of flowing blood in man. The end-point of thrombus formation is the time when the angular position of the head of the column of blood has shifted at least 4^{o} of arc from its original position. Subsequent investigators corroborated the fact that a thrombus with a platelet head was produced by this technique, and studied the effect of saturated and unsaturated fatty acids upon the thrombus formation time (Poole, J.C.F., Quart. J. Exp. Physiol. 44:377, 1959, Connor, W.E. and Poole, J.C.F., ibid 46:1, 1961, Connor, W.E., J. Clin. Investigation 41:1199, 1962).

The Chandler loop apparatus, as modified by Poole, will be used. I have had one of these instruments constructed, at my own expense, with 2 rotating heads so that determinations can be made in duplicate simultaneously. We have performed duplicate tests on 20 blood samples. The method is highly reproducible with a standard deviation of 0.45 minutes. We have compared the results on blood aliquots with silicone technique and with plastic syringes and pipettes, and no difference was found. We therefore propose to use plastic syringes and pipettes.

The smoking tests are done as follows: Patients are seen before breakfast and before smoking. Blood is drawn prior to smoking, directly after smoking and 20 and 60 minutes later. The thrombus formation time tests are run directly after the blood sample is obtained. Determinations of free fatty acids are subsequently made on serum aliquots and of triglycerides in some experiments. (The methods for the lipid determinations have been in use in my research laboratory for years. Free fatty acids will be determined using Dole's method, and triglycerides by the method of Van Handel and Zilversmit).

Although the findings in the first 60 subjects after smoking were statistically significant it is desirable that they be continued and extended. This will afford observations on all types of cigarettes and filters, and also on the effect of cigar and pipe smoking on thrombus formation times. In All further studies of cigarette smoking only one cigarette will be smoked in a normal manner. The results reported in my preliminary communication (J.A.M.A.-Sept. 20, 1965) were obtained after smoking two cigarettes. Larson et al (Tobacco. Experimental and Clinical Studies, Williams and Wilkins Co., Baltimore, 1961) have pointed out that in order to study the effects of smoking, ordinary and not forced smoking

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should be used. It is also planned to do repeat tests of the effect of smoking in the same individuals (at least 50) on different days in order to determine the consistency of the response. This is most important for several reasons: (1) It is necessary for the evaluation of drugs that may prevent the thrombotic effect of smoking and the determination of statistically significant results. (2) It is possible that determination of the thrombosing tendency of blood after smoking may lead to a standardized and better test procedure to detect those individuals more or less prone than others to the cardiovascular effects of smoking. It is also necessary to follow thrombus formation times after smoking for 1-4 hours in order to determine how long the effect of smoking persists.

The effect of intravenous or sublingual nicotine, and of I.V. epinephrine and norepinephrine, will be studied on hospitalized clinic patients at the Cedars of Lebanon Hospital. All studies will be in the postabsorptive state. The experimental design is as follows: An intravenous infusion of physiological saline will be started and several blood samples drawn over a half hour period to obtain control observations of fluctuations in the thrombus formation time and free fatty acids. Nicotine bitartrate will then be added to the infusion (2 mg. in a 10 minute period, to approximate the amount absorbed when 1 cigarette is smoked) and blood samples drawn after 5 minutes, at the end of the nicotine infusion, and 30 and 60 minutes later. In those patients in whom nicotine accelerates the thrombosis time it is planned to repeat the procedure another day, after phentolamine (Regitine) is given to see whether this epinephrine blockading agent interferes with the nicotine effect. Pulse rates and blood pressures will be recorded throughout. The use of nicotine I.V. with an initial period of normal saline infusion affords a better controlled study than can be done with smoking. A similar approach will be followed using intravenous epinephrine and norepinephrine. If epinephrine affects the thrombus formation time the studies will be repeated in the same individual ½ hour after the oral administration of 1 gram of nicotinic acid in order to evaluate whether blocking the free fatty acid release after epinephrine also blocks the effect on thrombosis.

All data obtained will be analyzed by the usual statistical tests for significance. In several experiments we have already found that epinephrine accelerates the thrombus formation time but more studies are needed.

The probable mechanism of the smoking effect on thrombosis is by way of the nicotine-induced release of catecholamines and the effect of the latter in enhancing platelet adhesiveness and platelet aggregation (J. R. O'Brien, Nature 200:763, 1963; J. L. Ambrus and I. B. Mink, Clin. Pharmacol. and Ther. 5:428, 1964; J. F. Mustard and E. A. Murphy, Brit. Med. J. 1:846, 1963). This indicates the study of drugs which either decrease catecholamine stores in the body (reserpine), or are adrenergic blockers of various types (propranolol mecamylamide, M. J. 1999, hexamethonium, bretylium, prometholol.) These drugs may prevent or diminish the effect of smoking on thrombus formation. The plan of evaluating these drugs would be to administer them at the usual clinical doses (several weeks for reserpine, several days for the adrenergic blocking agents) to subjects who had shown an accelerated thrombotic tendency after smoking and retest the effect of smoking after taking the drug. The drug would then be stopped and a second control

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smoking test taken after several weeks. It has been reported that prothrombin depressing drugs delay thrombus formation as studied in the Chandler rotating apparatus. The effect of smoking on thrombus formation times will therefore be tested in a group of patients who are taking oral anticoagulant drugs for coronary heart disease.

Mediation of the thrombosis-accelerating action of smoking via catecholamines suggests that stress and smoking may have a final common pathway in their relationship to myocardial infarction. In fact it is possible that when smoking relaxes tense individuals there may be a decrease in catecholamine release and catecholamine effects. Studies are therefore indicated of the relationship of tension and stress to thrombus formation. This is best done in non-smokers. Although there is some disagreement, it is probable that the measurement of urinary metanephrine and normetanephrine afford the best estimation of sympathetic nervous and adrenomedullary activities (U.S. von Euler, Clin. Pharmacol 5:398, 1964 and R. J. Wurtman, New Eng. J. Med. 273: 637, 1965). In these experiments blood would be drawn before breakfast and the morning urine sample analyzed for metanephrine and normetanephrine. The methods used by Westfall (T. C. Westfall, Effect of Nicotine and Nicotine Analogues on Tissue and Urinary Catecholamines in the Rat, Acta Physiol. Scand. 63: 77, 1965), will be followed.

We have observed that slightly less than half the cigarette smokers tested thus far (J.A.M.A., Sept. 20, 1965) did not have a shortened thrombus formation time after smoking. It would be important to know whether these individuals had a smaller catecholamine release after smoking than those subjects whose thrombus times did shorten on smoking. For this study urinary metanephrine and normetanephrine would be determined on the urine sample obtained at the end of the smoking test (usually one-half hour after smoking), the bladder having been emptied before smoking and a glass of water drunk at that time to guarantee adequate urinary output. The amine analysis would also be done on the urine sample obtained before the smoking procedure.

All data obtained will be statistically analyzed with the usual tests for significance. A full-time statistician is now available at the Cedars of Lebanon Hospital to help in this area.

Finally, an increase in platelet stickiness in half the subjects after smoking two cigarettes has been reported (Ashby et al, Lancet, July 24, 1965, Pg. 158). This is in accord with my published results. However the relation of platelet stickiness to glass to the thrombotic tendency of blood has never been proven. This is only an assumption. For this reason studies using the Chandler loop are preferable, since a true thrombus is produced in a plastic tube.

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6. Budget Plan:

a.	Salaries*	20, 588
b.	Expendable Supplies	1, 800
c.	Other Expenses	1, 200
d.	Permanent Equipment	1, 265
e.	Overhead (15% of a, b, c)	3, 538
	Total	$28, 391

*3 Technicians' salaries, including 9% fringe benefits.

7. Anticipated Duration of Work:

Three years.

8. Facilities and Staff Available:

My fully equipped biochemical research laboratory in the research building of the Cedars of Lebanon Hospital. I have been doing studies of fat transport for 14 years (see attached bibliography). There is adequate work space for 6 technicians but at present only 2 are employed. The lipid studies are supported by the U. S. P. H. S. Several residents at the hospital are available to work with me directly on the wards if funds are granted. Two additional machines for thrombus formation time determinations are now being built at my expense by the research building machine shop.

9. Additional Requirements:

The $1,265 under permanent equipment is for a Turner fluorometer.

Budget for second year is $28,310, and for the third year is $29,553.

(To the second and third years' budget a 5% increase in salary has been calculated for the three technicians, in accordance with institution policy).

10. Additional Information (Including relation of work to other projects and other sources of support):

This work has no relation to my other projects. I have no other support for this project on smoking and thrombosis.

100510171

Signature Herman Engelberg M.D.
Director of Project

Arthur E. Bell
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/ntwn0000

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Hyman Engelberg, M.D.

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

CONFIDENTIAL

February 10, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Little and Dr. Lynch.

FROM: Robert C. Hockett

SUBJECT: Renewal application of Paul S. Larson, Ph.D. and Herbert Silvette, M.D. No. 24R10.

We enclose herewith a renewal application with accompanying progress report from Drs. Larson and Silvette of the Medical College of Virginia. Correspondence with Williams & Wilkins relating to the cost of publication of the supplement is also enclosed.

It should be noted that the application includes a request for funds for the abstracting service as well as for the preparation of the manuscript and proof reading. In addition the forecast indicates the need of funds to make possible the publication of the supplement.

This application is being sent at present only to members of the committee, but we plan to include it in the agenda for the March meeting.

R. C. H.

1005101178

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

MISCELLANEOUS

#24-R-10

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

Activated: 11/1/54
Renewed: 8/1/57
and annually on
7/1 each year since

COMMITTEE:
Dr. Cattell, Chm.
Dr. Little
Dr. Lynch

Application For Research Grant

Date: January 19, 1966

1. Name of Investigator: Paul S. Larson and Herbert Silvette

2. Title: Haag Professor of Pharmacology and Visiting Professor of Pharmacology, respectively.

3. Institution & Address: Department of Pharmacology
Medical College of Virginia
Richmond, Virginia

4. Project or Subject: Abstracting and classifying the literature on the biologic effects of tobacco and preparation for publication of a Supplement to the Monograph on Tobacco

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

A copy of our progress report for the period July 1-December 31, 1965, which summarizes the status of preparation of manuscript for the Supplement, is attached. It also points out our decision not to adhere to 1964 as the cut-off point for material to be included and the reason for this. Inevitably, dealing in detail with post-1964 material at this time has somewhat slowed progress on the Supplement. None-the-less, we are near the half-way point and anticipate that a total draft will be completed by July or August. By draft is meant copy that can be expanded in situ with 1965-66 insertions as final draft for the printer, without appreciable retyping. It is fully expected that such expansion can be completed by the end of December and that a flow of chapters can start moving to the printer in October of this year. We regret the need to request this extension in time, but sincerely believe that the gain to the value of the Supplement will be rewarding.

Galley and page proof reading and index completion may go 6 to 9 months into 1967.

Copies of all new abstracts and coding of these will continue to be forwarded to The Council.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

Salary breakdown:

Dr. Silvette, full time	$14,000	a. Salaries	$ 20,576.
Secretary, full time	$ 4,512	b. Expendable Supplies	1,100.
Secretary, half time	$ 2,064	c. Other Expenses (Social Security)	554.
		d. Permanent Equipment	none
		e. Overhead (15% of a, b, c)	3,335.
		Total	25,565.

7. Anticipated Duration of Work: Six months for completion of manuscript plus 6-9 months for galley and proof checking and completion of indexing.

8. Facilities and Staff Available:

9. Additional Requirements: A subsidy for the publisher. Williams & Wilkins has estimated a cost between $15,000 and $20,000 based on some 525 pages and 1000 copies in the same format as the earlier Monograph.

10. Additional Information (Including relation of work to other projects and other sources of support):

It is our hope to continue this work, toward the goal of publishing a second supplement after an appropriate period, as well as preparing review articles as we have done in the past.

100510180

Signature Paul S. Larson

Director of Project

L. Daniel Cooke

Business Officer of the Institution

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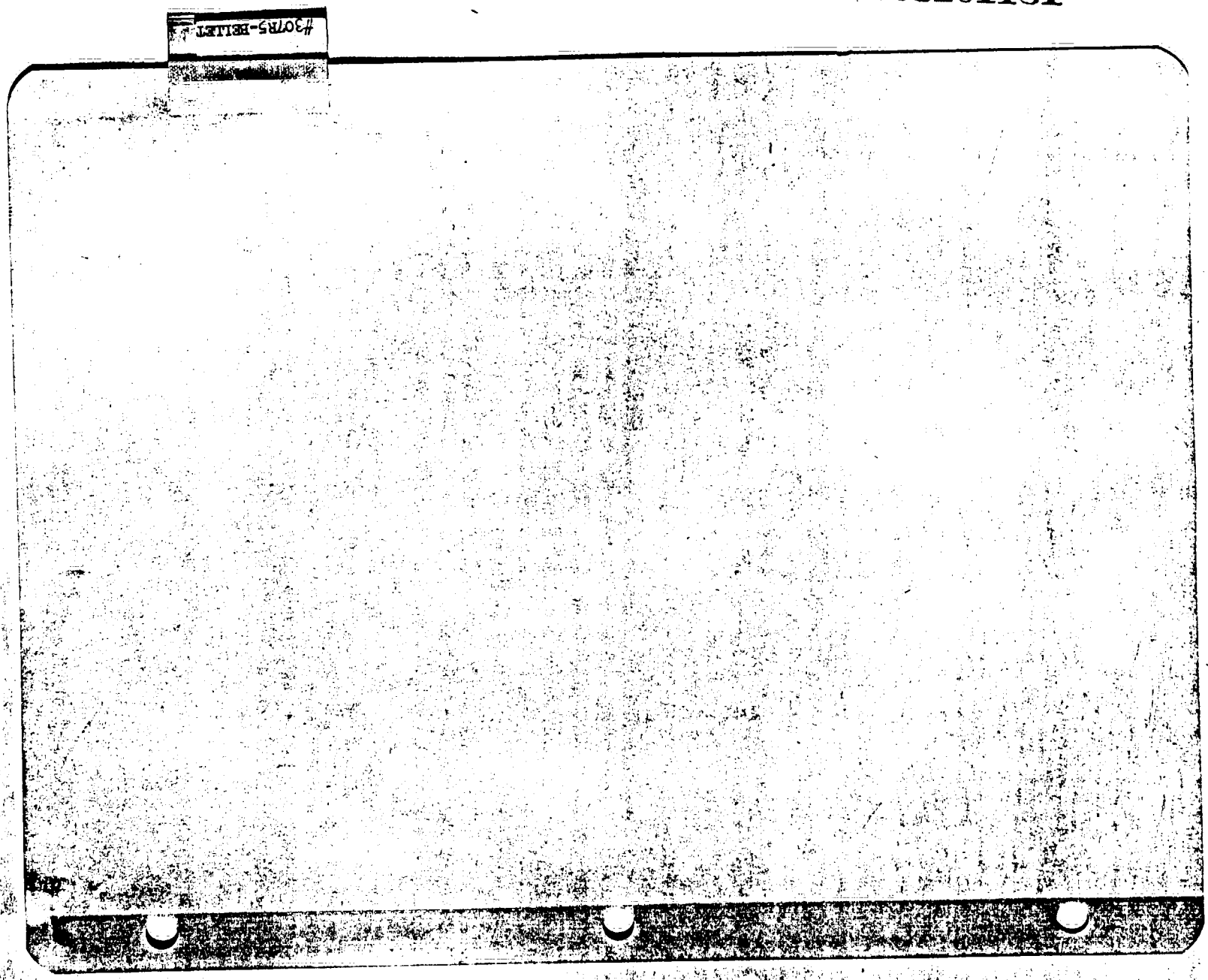

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH

CONFIDENTIAL

February 14, 1966

MEMORANDUM

TO: Committee comprising Dr. Cattell, Chairman, Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Samuel Bellet, M.D. - #307R5.

We enclose a renewal application, with progress report attached, from Dr. Samuel Bellet of the Philadelphia General Hospital. This would represent the tenth year of support for this research program. In each instance the anticipated duration of work has been stated as one year. Last year's grant was made without further commitment.

In addition to this application, another proposal has been received for the study of the effect of caffeine on blood lipids. Both will be included in the agenda for the March meeting.

R.C.H.

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CARDIOVASCULAR, PHARMACOLOGY, CHEMISTRY

#307R5

Activated: 7/1/61
Renewed: 7/1/62
" 7/1/63
" 7/1/64
" 7/1/65

Cf. #137
Activated: 11/15/56
Renewed: 11/15/57

Cf. #221
Activated: 1/1/5
Renewed: 7/1/6

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Application For Research Grant (RENEWAL)

Date: January 24, 1966

1. Name of Investigator: Samuel Bellet, M.D., Director, Division of Cardiology
Philadelphia General Hospital

2. Title: THE EFFECT OF NICOTINE ON VARIOUS PARAMETERS OF CARDIOVASCULAR FUNCTION

3. Institution & Address: Philadelphia General Hospital
34th Street & Curie Avenue
Philadelphia, Pennsylvania 19104

4. Project or Subject:
1. Determination of the Urinary Excretion of Nicotine After Smoking and Nicotine in Man and Dog.
2. Effect of Tobacco Substitutes and Special Filters on Catecholamine and Nicotine Excretion.
3. The Effect of Nicotinic Acid on Hyperlipidemia Induced by Tobacco Smoking and Nicotine.
4. Studies of the Effect of Smoking and Nicotine on the Metabolism of Triglycerides and Other Lipids.
5. The Effect of Nicotine on Experimental Atherosclerosis.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)
(see continuation sheets 1 and 2)

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$16,519.48
b.	Expendable Supplies	2,100.00
c.	Other Expenses	600.00
d.	Permanent Equipment	
e.	Overhead (15% of a, b, c)	2,802.92
	Total	$22,102.40

7. Anticipated Duration of Work: One Year

8. Facilities and Staff Available: Pages 3 and 4

9. Additional Requirements: NONE

10. Additional Information (Including relation of work to other projects and other sources of support):

NONE

100510184

Signature Vincent J. Dole & Ted

Director of Project

Benjamin F Burgess Jr

Business Officer of the Institution Benjamin F. Burgess, Jr.
Director of Research

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etailed Plan of roc edure:

. Determination of the urinary excretion of nicotine after moking and i nicotine in mah and dog,

sing a modific at ion of the method, of McNiven et al., icotine concentration in urine will be determined by means of as chromat ogr aphy. The extraction procedure and column now eing used has given us good reproducibility and satisfactory ecovery. The following studies will be made:

a) A comparis-a n of ni coti ne. excretion. wi'h cigarette, igar, and pipe smoking.

b) The effect of inhaling or not inhaling on nicotine xcretion.

(c) The effect of nicotinic acid given before smoking n nicotine excretion (see below).

d) The effect of special filters and tobacco substitutes n nicotine excretion compared to regular cigarettes (see below).

e) In anesthetized dogs, a comparison of cigarette, cigar, nd pipe smoke inhalation and intravenous nicotine on urinary icotine concentrations.

reliminary studies have shown significantly greater nicotine excretion with cigarette smoking than cigar or pipe smoking which is reversed when cigars and pipes are inhaled and igarettes not inhaled.

Effect of tobacco substitutes and special filters on catecholamine and nicotine excretion.

n a previous study (see Progress Report) it has been hown that smoking charcoal filter cigarettes and cigarettes containing pipe tobacco have a mobilizing effect on free fatty cids similar to that of standard cigarettes. Cigarettes ontaining shredded lettuce leaves had no effect on free fatty cids.

n the projected study, this investigation will be extended and the excretion of catecholamines and nicotine with he various cigarette forms will be measured and compared.

. The effect of nicotinic acid on hyperlipidemia induced by obacco smoking and nicotine.

here has been considerable interest in recent years in he effect of nicotinic acid (NA) on certain aspects of lipid etabolism. NA reduces plasma levels of FFA and prevents the FA rise which follows epinephrine and norepinephrine. This nterested us in a study of the effect of NA on the lipid effects of smoking and nicotine in human subjects and dogs.

ubjects will smoke 2 cigarettes or an equivalent amount f cigar or pipe tobacco andcontrol curves of the FFA response. ill be obtained. The experiments will be repeated on a different day with subjects smoking 30 minutes after ingestion. f 200 mg of NA. Preliminary results have shown a fall in asting serum FFA concentration and an inhibiting effect on. he rise after smoking. To determine if the FFA effect is econdary to an effect of NA on catecholamine secretion, urinry catecholamines will be measured during 4-hour periods of

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BUDGET PLAN:

a. Salaries

Chief - Laboratory (Part-time)	$ 3,616.00
(2) Medical Laboratory Technicians (Full time)	10,934.00
Social Security	761.45
Workmen's Compensation	56.17
Fringe Benefits (Full-time only)	320.00
Pension	831.86
	$16,519.48

b. Consumable Supplies

Dogs, their board, keep, & medical care (Approx. 60 dogs)	$ 1,500.00
Radioactive Isotopes, Enzymes	200.00
Chemicals, Catheters, ECG & Recording Paper	300.00
Glasswame, Instruments, Syringes, Needles, etc.	100.00
	$ 2,100.00

c. Permanent Equipment

NONE

d. Overhead (15% a,b, & e)	$ 2,882.92
e. Travel - National Meetings, Presentation of Papers	$ 200.00
Repairs	100.00
Photography & Reprints	300.00
	$ 600.00
GRAND TOTAL	$22,102.40

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smoking cigarettes, cigars or pipes with and without NA. To determine the effect of NA on nicotine availability in the body, the nicotine content of the urine collected during 4-hour smoking periods, with and without the ingestion of NA, will be compared.

In dogs under chloralose anesthesia, smoking will be achieved by tracheal intubation. After obtaining control FFA responses to 2 gm of cigarette, cigar, pipe tobacco smoke or equivalent I.V. nicotine, the experiments will be repeated following I.V. administration of 100 mg of NA. The effect of NA on the rise in serum triglycerides which follows smoke inhalation and nicotine (see Progress Report) will also be determined.

4. Studies of the effect of smoking and nicotine on the metabolism of triglycerides and other lipids.

Studies in the human and dog have been initiated to determine the effect of smoking and nicotine on the synthesis and metabolism of tri-, di-, and monoglycerides, cholesterol, cholesterol esters, and phospholipids. Labeled precursor palmitic acid will be administered and the incorporation of the isotopic fatty acid into lipoprotein lipids will be determined by column and thin-layer chromatographic separation and liquid scintillation spectrometry. The effect of smoking and nicotine on the rate and degree of fatty acid incorporation will be studied. These studies should help clarify the mechanism of the rise in serum triglycerides which follows smoking and nicotine in dogs (see Progress Report).

5. The effect of nicotine on experimental atherosclerosis.

Long-term studies are to be done on dogs to determine if the hypercholesteremia produced by nicotine (see Personal Publications--#1) will have any atherogenic effect when the animals are maintained on a human-type diet (high fat-cholesterol) or are subject to induced experimental atherosclerosis.

Forty dogs will be divided into 4 groups of 10. The first 2 groups will receive a balanced laboratory dog ration. One group will receive daily injection of nicotine in a slow absorption vehicle (see Personal Publication--#11); the other will serve as a control. The second 2 groups will receive the same diet supplemented by 10 gm of cholesterol and 50 gm of butter. One will receive nicotine, the other will be a control group. Half of the dogs will be sacrificed at 3 months and the remainder at 6 months for pathologic study. Serum lipid analyses and electrocardiographic studies will be made at intervals.

Twenty additional dogs will be given a medical thyroidectomy by the administration of radioactive iodine (I-131), followed by 2 gm daily doses of propylthiouracil. All of the dogs will receive the cholesterol-butter supplemented diet plus 3 gm of cholic acid daily. Nicotine will be given to 10 dogs and 10 will serve as controls. Studies and sacrifice will be as described above.

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acilities and Staff A ai-lable:

1) General Faci lit iies--

. A 2:,50b bed municipal y owned and operated general ospital staffed by the faculties of 5 medical schools, both ul1-time. and part-time. A large percentage of the beds are sed for cardiovascular cases. Specialty clinics which are taffed and used by the Division of Cardiology include an dult and a pediatric heart cliinic, hypertension clinic, eripheral vascular clinic and diabetic clinic among others. he Division of Cardiology is responsible for all problems elating to the subject of cardiovascular disease for the ntire hospital.

. The Division of Cardiology consists of:

. A heart station equipped with complete instrumentation for cardiovascular diagnosis: Electrocardiography, Radioelectro-ardiography, Phonocardiography, Vecctorcardiography, Ballisto-ardiography, Fluoroscopy (Image Intensifier) , Cardiac atheterization, Angiography, Peripheral Vascular Diagnosis· Constant Temperature Room), Library and Reading Room.

: A biochemical laboratory devotedifully to cardiovascular esearch (distinct from the hospital clinical laboratory) . t is equipped for doing lipid analysis, catecholamine analysis, lood. gas analysis; flame photometry, electrophoresis, smometry, spectrophotometry, colorimetry, chromatography, olarography, etc.

. An Isotope Laboratory for radioactive tracer studies

4. An Animal Laboratory

. An Enzyme and Drug Evaluation Laboratory

. A Pulmonary Function Section

2) Major Items of Permanent Equipment (Biochemical Laboratory):

. Well-type scintillation counter, scaler, probe, rate meter nd pulse height analyzer (NRD) .
. A vibrating reed electrometer (Nuclear-Chicago) with slide hamber, glassware, lonization chambers for measuring eak Beta radioactivity of Tritium, Carbon-14, and ulphur-35, in solid, liquid and gaseous phase.
c. Ultracent ifuge
. Refrigerator centrifuge
. Fraction collector.
. Column chromatography equipment
. Gas chromatography equipment
. Paper electrophoresis equipment
itometer ovolt) j. ctrop ter e m
. Fluorilmeter (Farrand) i. Photometer (flame)
. Colorimeters (Evelyn-Klett) n. Osmometer (Fiske)

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

o. Van Slyke volumetric and manometric apparatus
p. Scholander respired air analyzer
q. pH meters (Beckman Model G and Metrohm)
r. Polarograph
s. Respirometer
t. Dual count rate meter, recorder and scintillation counter combination

(3) Staff--

Director of the Division
Section Chief: Section of General Biochemistry
Section of Radioisotope Studies
Section of Adult Hemodynamics
Section of Pediatric Hemodynamics
Section of Peripheral Vascular Disease
Clinical Section
Section of Drug Biochemical Laboratory
Section of Animal Research

5 Attending Physicians
1 Electronic Engineer
10 Residents & Fellows in Cardiology
2 Technicians
5 Laboratory Technicians

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

#3LOR5-BLNG

1005101190

Source: https://www.industrydocuments.ucsf.edu/docs/nlwh0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A. February 15, 1966

CONFIDENTIAL

MEMORANDUM

TO: Subcommittee comprising Dr. Cattell, Chm., Dr. Lynch and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Richard J. Bing, M.D. - No. 310R5.

We enclose a renewal application accompanied by a progress report from Dr. Richard J. Bing of Wayne State University, Detroit, Michigan.

Dr. Bing has applied on an annual basis and has rendered a comprehensive report at the end of each year. Ten reprints acknowledging Council For Tobacco Research support have been mailed to members of the Board during the past year.

Our budget projection includes provision for maintenance of this project at substantially the current level.

This proposal is being sent at present only to members of the committee but it will be included in the agenda for the March meeting.

R. C. H.

1005101191

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

ARDIOVASCUIAR HARMACOLOGY and CHEMISTRY

310R5

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enewed: 7/1/62
Renewed: 7/1/63
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f. 1
1 55 to 7/1/59
f. #270
1/60 to 7/1/61.

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
r. Cattell, Chm.
Dr. Lynch
Dr. Jacobson

Renewal
pplication For Research Grant

ate: February 10, 1966

L Name of Inveshgator: Richard J. Bing, M.D.

2. Title: rofessor and Chairman, Department of Medicine

Institution & ddress: ayne State University School of Medicine
400 Chrysler Freeway
etroit, Michigan 48207

Projeu or Subject: he Determination of Rapid Changes in Coronary Blood Flow
ith Coincidence Counting; A Correlation of Coronary
emodynamics to Cardiac Contractility.

5 Detailed Plan of Procedure (Use additional pages if more space is required.)

lease see attached.

100

1

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$ 6,149.00 *
b.	Expendable Supplies	4,000.00 **
c.	Other Expenses	---
d.	Parmanent Equipment	---
e.	Overhead (15% of a, b, c)	1,522.00
	Total	11,671.00

*Includes $236.50 FICA and $412.50 Retirement (7-1/2%)
**To partially defray cost of the isotope, Rubidium-84.

7. Anticipated Duration of Work:

One year

8. Facilities and Staff Available:

Laboratory facilities and staff are available at 645 Mullett and Detroit General Hospital.

9. Additional Requirements:

10. Additional Information (including relation of work to other projects and other sources of support):

100510193

Signature

Director of Project Richard J. Bing, M.D.

Clifford M. Van Buskirk

Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Detailed Plan of Procedure

As the progress report has indicated, a method has been developed which measures quantitatively coronary blood flow over a period of time. The main disadvantage of this procedure is that only overall figures of coronary flow over periods of 8 minutes can be obtained, and that rapid changes in coronary blood flow cannot be assessed. To overcome this difficulty, it is proposed to add to this technique a modification patterned after the method of Donato in which myocardial perfusion in man can be evaluated with rubidium by means of precordial counting. Donato's difficulties arose from the use of singles rather than positron emitters; therefore, extremely difficult collimation and complex machinery had to be used. However, his method is applicable to our instrument if the slow counting system is replaced with a rapid counter. The method of Donato is based on the principle that when a single bolus is intravenously injected into a patient, the initial partition of the bolus of rubidium is proportional to the distribution of cardiac output and that the distribution is maintained for a certain time after completion of the first circulation. On these grounds, it has been found that the myocardial uptake of rubidium immediately after a single injection furnishes an approach to the problem of estimating myocardial blood perfusion. The formula for the calculation of myocardial blood flow by the bolus method is given as follows:

$$MCK(R) = \frac{D_f \times F \times 60}{\int_0^{\infty} R_k(t)\, dt} \times 100$$

1005101194

where the denominator of this equation is represented by the area under the curve during one passage of the radioactive material through the heart chambers (see enclosed Figure 1). This occurs within 10 to 15 seconds. It is for

Source: https://www.industrydocuments.ucsf.edu/docs/hlwn0000

his reason that a very rapid response recording system has to be installed n order to measure these rapid changes. The details of the calculations coronary flow have been discussed in Donato's original paper. (Circulation 9:195, February, 1964)

he coincidence counting system would eliminate two difficult tions of this method: (1) the complicated collimation, and (2) the extremely bersome correction for activity in chamber blood; to accomplish this, it s necessary to separate radioactivity in the cardiac chambers from that of he heart muscle by preceding injection of radioiodinated albumin Thus he ravascular component can be calculated only if the radioactive concentration n blood is measured, and this is accomplished by the injection of radio- dinated serum albumin, referred to above. The apparatus, therefore, onstructed and used in our laboratory, is directly applicable to the nstantaneous measurement of coronary blood flow without further modification th th excep i n o sta l t n o rap d: ecor i g v e. It uld be stressed that the use of the coincidence counting system greatly plifies the whole procedure.

sing this method in combination with our previously developed ethod which continuously determines coronary blood flow for periods ranging rom 6 to 15 minutes, it will now be possible to study acute and immediate ffects of drugs acting on the coronary circulation such as nicotine and itroglycerin. In the past, such studies could not be performed in man because both the nitrous oxide method and the rubidium-84 method measure nly slow and non-phasic changes in coronary blood flow.

he second part of this project deals with the measurement of ardiac contractility in man as correlated with the measurement of the

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

instantaneous effect of nicotine and other drugs affecting coronary blood flow. This work is based on the construction of a new instrument which enables the direct measurement of cardiac contractility in man. In principle, this instrument is a catheter with two separate receptor wires which can be pushed through the distal lumen of the catheter and inserted into the myocardium to a distance of about 1 to 1.5 millimeters. These two wires are connected to a Wheatstone bridge which measures the distance between the two wires and therefore determines the shortening of the heart muscle from the inside of the ventricular chambers. The catheter has been constructed by General Motors and accomplishes direct measurement of cardiac contractility without exposure of the heart. Using this catheter, one is in a position to measure the effect on cardiac contractility of various pharmacological agents.

It has become obvious that antianginal drugs studied here act in a similar manner; they increase coronary blood flow in normal individuals, but have no effect in patients with coronary artery disease; they all produce a diminution in cardiac contractility. This latter may be the result of direct effect on the heart muscle or it may be a peripheral effect. Using these two new methods, the coincidence counting system and the new Wheatsone bridge catheter, it may now be possible to study the effect of coronary vasodilators on both cardiac contractility and on coronary blood flow, and possibly also on myocardial metabolism, the latter being accomplished by intubation of the coronary sinus. These new procedures also would give some information on the effect of nicotine on the human heart. The questions that could be answered pertain to the temporary relationship between the effect of nicotine on the heart muscle and on the coronary arteries.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnkm0000

The final contemplated project is a continuation of the work dealing with the epidemiological studies concerned with the recognition of coronary artery disease. It is proposed that the rubidium-nitroglycerin test should be carried out in the detection of the coronary prone patient and in the differential diagnosis of coronary suspected patients. This test is based on a difference in response to nitroglycerin of normal hearts and of arteriosclerotic hearts. It is proposed that two groups of individuals be studied, the first consisting of patients which are coronary prone. The other group will be composed of patients who have vague complaints which point towards the presence of coronary artery disease but in whom there has been no definite clinical evidence for this. The line of approach in these two groups of patients is identical: determination of the coronary profile, serum cholesterol, lipids, electrocardiographic changes, body build, personality, social factors, and particularly the rubidium-nitroglycerin test and coronary arteriography.

The significance of the proposed research may be summarized as follows:

The determination of instantaneous changes in coronary blood flow with a single bolus injection method. This procedure, as combined with the coincidence counting method and the positron emitting material, Rubidium-84, will enable us to study instantaneous changes in coronary blood flow as produced by nicotine or coronary vasodilator drugs. Such a procedure has not been possible in man heretofore. It should be mentioned here that this method has already been started using our Co-Insitron machine at the University of Indiana under Dr. Hickam's direction. The questions that can be posed are: What is the immediate effect of nicotine on the coronary blood flow? How long does the effect last? and How large an increase in coronary blood flow is observed at various intervals following either the smoking of cigarettes or the injection of nicotine.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwx0000

The second problem, the determination of cardiac contractility, combined with the measurement of coronary blood flow, may be of help in determining the direct effect of nicotine, of beta ganglionic blockers, and of nitroglycerin and other similar compounds on the contractility of the heart muscle. Heretofore, this has been only possible by indirect means such as the determination of the pressure rise during the systolic phase of the cardiac cycle or the tension time index. The questions that can be answered are concerned with the time relationship between the coronary dilatation as observed with nicotine and cardiac contractility.

The third part of the project consists in a continuation of the work previously proposed dealing with long range studies in the definition of coronary profile as influenced by smoking, genetic factors, hypertension, etc.

To summarize, the proposal is consistent with new approaches to cardiac physiology with special reference to clinical pharmacology, approaches which are based on the development of new techniques as applied to man.

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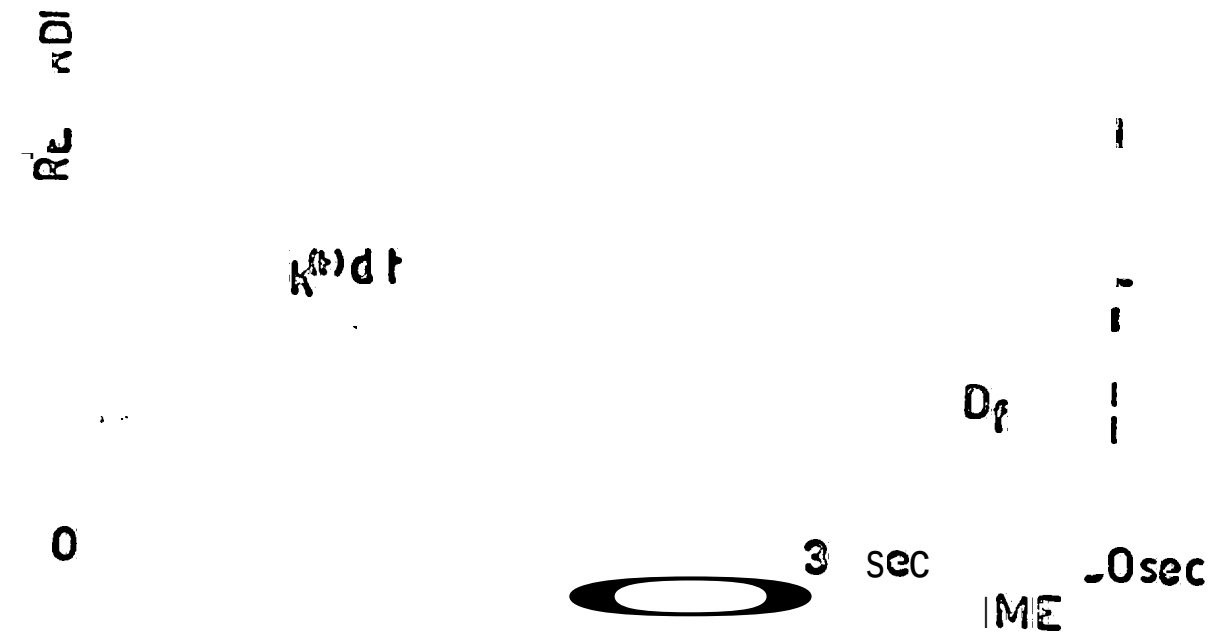
Source: https://www.industrydocuments.ucsf.edu/docs/nlwn0000

RECORDING WITH RATEMETER



Figu 1

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

#362R3-MANOS

10051012000

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

CONFIDENTIAL

February 14, 1966

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chairman, Dr. Lynch and Dr. Little.

FROM: Robert C. Hockett

SUBJECT: Renewal application from John P. Manos, M.D. - #362R3.

We enclose herewith a renewal application with accompanying progress report from Dr. John P. Manos of the Medical College of South Carolina.

Dr. Kotin questioned the likelihood of this study yielding significant results and, at the time of the last renewal, Dr. Manos was informed that it represented a terminal grant for that project.

This application is being sent at present only to members of the committee, but we plan to include it in the agenda for the March meeting.

R.C.H.

RCH:ek

encl.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

VIRUS, Previous Infection

#362R3
Activated: 5/1/63
Renewed: 5/1/64
Renewed: 5/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Lynch
Dr. Little

Renewal

Application For Research Grant

Date: January 31, 1966

1. Name of Investigator: John P. Manos, M. D.

2. Title: Associate in Microbiology

3. Institution & Address: Medical College of South Carolina
80 Barre Street, Charleston, South Carolina 29401

4. Project or Subject:
The separation of the various subcellular neoplastic particles by ultrafiltration methods and their characterization in laboratory animals and in tissue cultures of various cell lines.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Please see Progress Report #4 attached to application.

1005101202

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a. Salaries	600 00
b. Expendable Supplies	000
c. Other Expenses	1000 00
d. Permanent Equipment	1000.00
e. Overhead (15% of a, b, c)	440.00
Total	12 0.00

7. Anticipated Duration of Work One year.

8. Facilities and Staff Available:

Millipore filter apparatus and filter,, Gelman centrifuge filter holders and filters, various centrifuges, Serval regrigerated super-sneed centrifuge, spectrophotometers, Micro Kjeldahl equipment, photographic equipment, chromatogranhic apparatus, sonic oscillators, fluorescent microscopy equipment, electron.microscopy and other equipment pertinent to microbiological work. Consultation and technical assistance from various Medical School Departments.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of support):

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH

CONFIDENTIAL

CTR GRANT #362

PROGRESS REPORT NO. 4

JOHN P. MANOS, M.D.
Department of Microbiology
Medical College of South Carolina
80 Barre Street
Charleston, South Carolina

May 1, 1965 - May 1, 1966

THE SEPARATION OF THE VARIOUS SUBCELLULAR PARTICLES BY ULTRAFILTRATION METHODS AND THEIR CHARACTERIZATION IN LABORATORY ANIMALS AND IN TISSUE CULTURES OF VARIOUS CELL LINES.

1. As indicated in Progress Report #3, 480 one month old A/J mice (Jackson Memorial Laboratories) were inoculated intra-nasally with fractions prepared from lung adenomas derived from A/J mice of at least one year of age. The mice were inoculated in groups of 24 with 10 fractions derived from the adenomas and 10 fractions derived from lungs of mice with little or no incidence of lung tumors (C57BL/6J Jackson Memorial Laboratories). The mice have been under observation for 10 months. It is planned to observe them for about 16-18 months at which time they will be sacrificed and lung tumor counts will be made. The endpoint of this experiment is to look for an increased number of mice with lung tumors in those inoculated with adenoma extracts and/or for an increased tumor count per lung per animal in the test animals as opposed to the controls.

2. The various fractions, both from the adenomas and normal lung have been serially passed into Mouse Kidney and Mouse Lung tissue cultures and have so far been passed blindly seven times. Each passage is being observed for two weeks then challenged with Coxsackie B-3 virus in hopes of detecting interference. So far, neither of the two mouse cell lines have shown any evidence of cytopathogenicity, toxicity or viral interference. It is planned to carry out the blind passages about a dozen times and then proceed with testing the various fractions in Mouse Embryo and Mouse Carinoma cell lines.

3 A human carcinoma of the lung was obtained as a surgical specimen and a portion of fresh lung at autopsy from a patient who died of a non-pneumonic process. Both of the human lung specimens were broken up into the various fractions as previously outlined and a portion of each fraction was inoculated into paired rabbits with the use of Freund's complete adjuvant. After an appropriate immunization series the animals were exsanguinated. The human lung fractions and their respective rabbit antisera, ten against normal lung fractions and ten against lung carinoma fractions, are now being studied for antigenic differences and to see if a specific antigen(s) or lack of a specific antigen(s) may be detected peculiar to the lung carcinoma. Methods of approach are gel diffusion techniques, tanned cell agglutination methods and possibly complement-fixation evaluation. We have presently embarked on standardizing double diffusion techniques as the first approach to the study of the antigenic characterization of these fractions.

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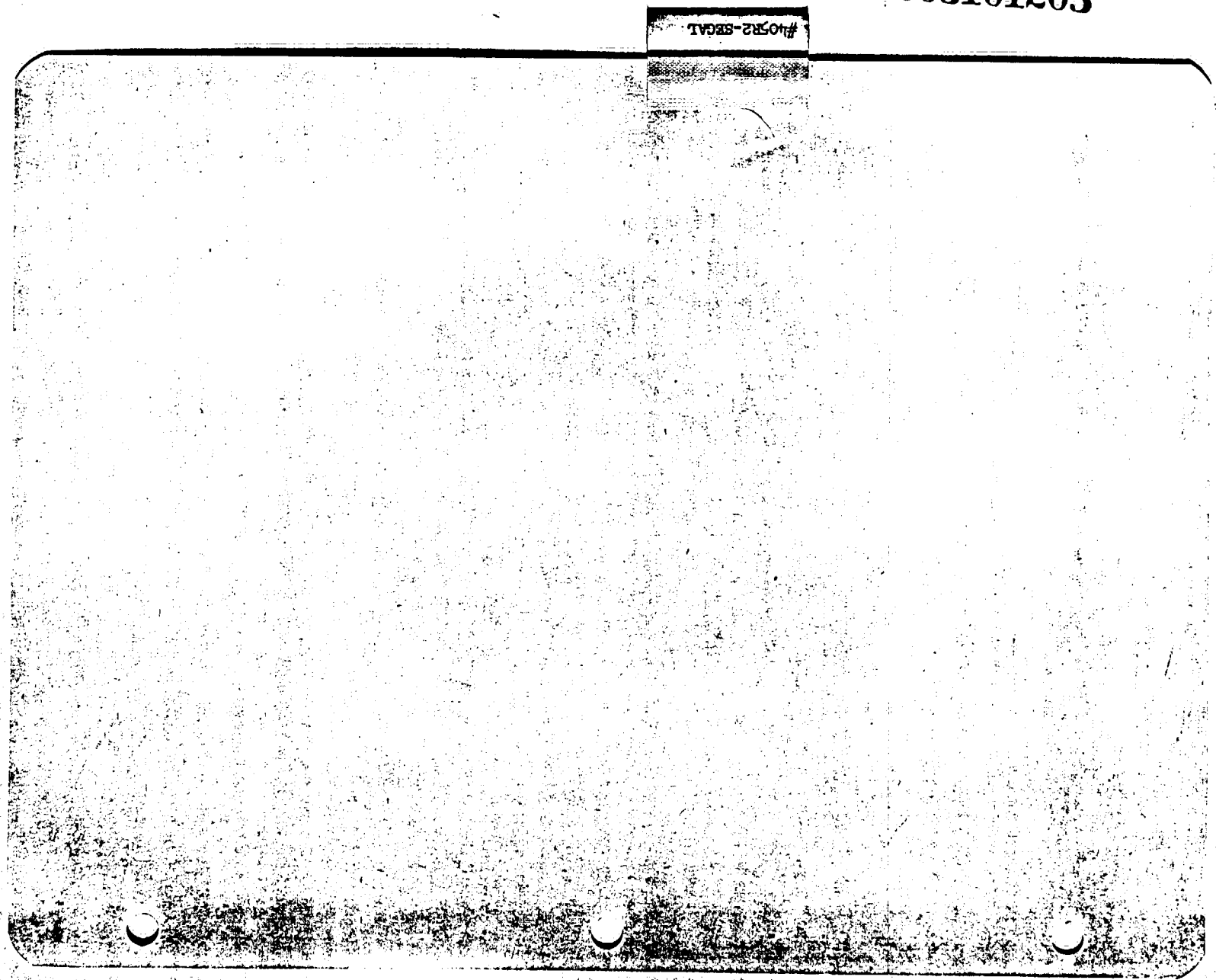
Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

1005101205



Source: https://www.industrydocuments.ucsf.edu/docs/nlwh0000

HE COUNCIL FOR TOBACCO RESEARCH, U. S. A. C AL ebruary 16, 1966

EMORANDUM

O: The committee comprising Dr. Reimann, Chm., Dr. Lynch and Dr. Jacobson.

FROM: Robert C. Hockett andiVincent F. Lisanti

UBJECT: Renewal application from Maurice S. Segal, M.D. - No. 405R2.

Enclosed is a renewal application accompanied by an outline progress eport covering the first eighteen months of project activities from Dr. Maurice . Segal of the Boston City Hospital (Tufts) Lung Station, Boston, Massachusetts.

his renewal application is for the final year of a three year award, Which was initiated on July 1, 1964.

he activities of Dr. Segal and his associates especially in the last ear or two have been both productive and meritorious. They have not only per- ormed as pro posed but have exceeded their overall program by the introduction of ew parameters for evaluation.

he new facilities have been visited by both staff members and were ound to be beyond our expectations.

h private and Federal funds have made the facilities and equipment ossible.

ecause of the complexities of the problem area and the need for more learly defined terms the Scientific Advisory Board should anticipate continued pport for additional periods of time, certainly three and perhaps six years.

t seems that the productivity and clarification of these problem areas will assist the medical profession and contribute to the knowledge of the role hat smoking plays.

R. C. H.

. F. L.

0051 06

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

PATHOLOGIC-DIAGNOSTIC
(Pulmonary Disease)

#405R2
Activated: 7/1/64
Cf. #2
1/1/55 - 1/1/57
Cf. #198
7/15/58 - 7/15/59

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Reimann, Chm.
Dr. Lynch
Dr. Jacobson

Renewal
Application For Research Grant

Date: February 9, 1966

1. Name of Investigator: MAURICE S. SEGAL, M.D.

2. Title: Clinical Professor of Medicine

3. Institution & Address: The Boston City Hospital
Lung Station (Tufts)
818 Harrison Avenue
Boston, Massachusetts 02118

4. Project or Subject:

CHRONIC BRONCHITIS ENTITIES

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

The Aim is to carry out an integrated study of ancillary and newly developed parameters to delineate the different types within the chronic bronchitis syndrome and their evaluation. The following procedures will be used:

1. The history and clinical condition of the patient

2. a) The physiological abnormality as measured by pulmonary function studies
and
b) blood (cellular and chemical) analyses

3. Sputum evaluation to include: physical, cytological, histochemical, chemical and bacteriological characteristics

4. Radiological evaluation by means of fluoroscopy, chest X-ray, bronchography and laminography

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

5. Detailed Plan of Procedure:

A. Clinical Material

Patients to be studied in this project will be recruited from the Research Clinic at the Lung Station (Tufts), Boston City Hospital. Patients with "non-specific" chronic bronchitis, including the widest possible clinical spectrum, ranging from the young bronchitic through the terminal stages of bronchitis, emphysema and cor pulmonale, will be selected. We would estimate that a total of 200 to 300 patients will be studied over the full 3-year period.

"Non-specific" bronchitis for the purpose of this study will be defined as patients having chronic productive cough which has been present for at least 1 year, associated or not with non-paroxysmal wheezing and exhibiting signs of chronic air flow obstruction by either clinical, radiological and/or laboratory assessment.

Clinical, laboratory and X-ray evaluations will be performed at a time when the patients are free of acute exacerbations of bronchitis and/or evidence of clinical circulatory failure.

To attain this state, the patients will receive an intensive treatment designed to obtain maximal bronchial cleansing and bronchodilatation. This measure is designed to obtain at least 2 sets of clinical, physiological and sputum data and 1 adequate radiologic evaluation within the assumed "basal" condition period in order to standardize the method of data collection.

Repeated sets of studies will be done with at least 1-year intervals to follow the progression of disease. The patients will be evaluated clinically (and laboratory-wise) during this period to document their clinical course.

B. Methods of Evaluation

1. The Clinical Evaluation will include:

A detailed clinical history and physical examination, with particular emphasis on the character, duration and progression of symptoms. Etiological factors, such as smoking, history of acute diseases and respiratory offenders will be particularly stressed. This will include a clinical gradation of the level of bronchial inflammation which we have standardized at our Clinic.

2. Pulmonary Function Studies:

Each patient will have a basic profile of pulmonary function including spirometry, intrapulmonary mixing and respiratory gas

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0009

exchange studies. Arterial blood studies will include: Po_2, Pco_2 and pH.

3. Sputum:

The patient will bring in a complete 24-hour sputum collection in a clean glass container. The examination will include: a) Gross description of its physical characteristics; b) Volume; c) Number of cells per ml. of sputum will be directly determined in a hemocytometer; d) Cover-slip smears of representative areas will be stained by Papanicolou's method for differential counts of the sputum cells. The following cell types will be differentiated: histiocytes (with emphasis on types of phagocytized materials), giant cells, bronchial epithelial cells (with emphasis on metaplasia and neoplasia), neutrophils (PMN's), eosinophils, lymphocytes, monocytes and plasma cells. The cytological data will be calculated to give the percentage of each cell type in the sputum. The total number of each cell type excreted per day will be calculated on the basis of this percentage, the sputum volume and the number of cells per ml.; e) A portion of a representative sample of the patient's sputum will be studied to determine the protein constituents of the sputum, using methods developed in this laboratory. Electrophoretic methods will be utilized, using fractions of the sputum separated by physical and chemical means. The sputum protein nitrogen content will be determined, using the Biuret method; f) An aliquot of the thickest portion of each specimen will be evaluated for adhesiveness and viscosity by methods developed in this laboratory; g) The remaining portion of the sputum will be cultured for bacterial pathogens, and comparable gram stains will be evaluated. Colony counts of the pathogens will be carried out, using the methods described by Laurenzi (Am. Rev. Resp. Dis., 86:106, 1962).

4. Radiological Studies:

a) Each patient will first have a set of conventional plain chest X-rays in full inspiration and expiration in the P-A position and at least in one lateral position.

b) Bronchography: Using a standard conventional procedure, the smallest possible amount of aqueous contrast media will be instilled to obtain a coating picture of the entire bronchial tree. P-A, oblique and lateral views will be obtained. The first stage of this procedure will entail a preliminary standardization of the technique in this group of patients with excessive cough and expectoration. Subsequently, bronchography will be performed in all the clinical ranges of chronic bronchitis with and without clinical evidence of established emphysema, secondary to chronic bronchitis.

c) Chest laminography will be added only in cases of chronic bronchitis with established emphysema for the complementary visualization of the vascular abnormalities (see 10, A-3).

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwx0000

5. Pathology:

The facilities of the available general Pathology Department are not geared to complement the purposes of this project. We are, therefore, developing a section devoted to the specialized techniques required for lung pathology. The initial purpose of this section would be to set up methods for whole lung fixation and for the critical evaluation of bronchial biopsies as applicable to the patients in the study.

6. Methods of Analysis:

The method of collecting data has already been developed in cooperation with a statistical consultant. Processing and evaluation of data are still under consideration. It is expected that a final decision will be made during the the third year of the project when sufficient data is on hand for a valid projection.

Our goals will be directed toward:

a) Ascertaining the role of potential etiologic factors (e.g., infection, smoking, allergy, etc.)

b) The evolutionary pattern under well-controlled treatment

c) The determination of our ability to classify varieties of the chronic non-specific lung syndrome

d) Finally, evolving a suggested standardized terminology--classification of chronic obstructive lung disease

6. Budget Plan:

a)	Salaries	$ 42,188.00
b)	Expendable Supplies	3,500.00
c)	Other Expenses	1,000.00
d)	Permanent Equipment	1,500.00
e)	Overhead (15% of a,b,c)	7,003.20
	Total	$ 55,191.20

7. Anticipated Duration of Work:

One more year within scope of present protocol; however, it would appear that 3-5 more years will be required.

8. Facilities and Staff Available:

A. Facilities:

The clinical, physiological and sputum studies will be entirely

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

performed in the new expanded area of the Lung Station (Tufts). The Station is presently equipped with the basic major laboratory facilities necessary for this study. These include:

1. Equipment for pulmonary function studies: spirometers, multi-channel recorder, fast response gas analyzers for CO_2, N_2 and CO; electrodes for direct determination of blood PO_2, Pco_2 and pH.

2. Equipment for sputum studies: Complete setup for cytological and histochemical studies; binocular microscopes with microphotographic attachment and water bath. Bacteriological setup including inoculating room, autoclave, 2 incubators, media preparation facilities, etc.

3. Trained technicians

4. X-ray equipment: a new 300 Ma Keleket unit plus the facilities of the Central X-ray Department of the Boston City Hospital, including a special picker laminographic attachment and ceiling tube mount.

B. Staff

Maurice S. Segal, M.D.; Clinical Professor of Medicine (Principal Investigator)

Mauricio J. Dulfano, M.D.; Assistant Professor of Medicine (Physiology)

Sanford Chodosh, M.D.; Assistant Professor of Medicine (Bacteriology and Histochemistry)

Ernest J. Ferris, M.D.; Assistant in Radiology

James A. Shaka, M.D., Associate Professor of Pathology

Research Fellow; Ear, Nose and Throat Specialist (on fee basis)

Physiatrist (Pulmonary Physiotherapy) (on fee basis)

Technician; Bacteriology and Cytology

Technician; Pulmonary Physiology

Technician; Sputum Chemistry

Secretary

9. Additional Requirements:

-None-

10051011

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0199

10. Additional Information (Including relation of work to other projects and other sources of support):

A. Previous Work Done on this Project and Related Fields

1. Clinical Studies:

During the past 10 years, our Station has accumulated a tremendous experience in the chronic bronchitis syndrome, and certain patterns are slowly emerging. These tentative patterns can be summarized as follows:

a) Progression of Symptoms

1. Chronic, episodic cough and expectoration, with interval-free periods.

2. Chronic, steady cough and expectoration

3. Chronic, progressive cough and expectoration

b) Sputum Characteristics

1. Mainly inflammatory mucosal involvement (indicated by predominance of cellular over glandular elements. The sputum is comparatively scanty).

2. Mainly mucid (bulky and "thicker")

3. Scanty, but tenacious expectoration (different physicochemical mucous character)

4. Combination of above

c) Syndrome Patterns

1. Starts as classic allergic bronchial asthma and progressively the bronchitis symptoms and signs supercede the asthmatic ones.

2. Starts with acute respiratory infection and over a short period of time, usually months, develops into severe ppogressive "asthmatic bronchitis".

3. Starts with shortness of breath predominantly and over the years develops progressive bronchitis signs with or without progressive shortness of breath.

4. Starts as specific endobronchitis (e.g., tuberculosis) and progresses into non-specific chronic bronchitis, even after the original reason has been controlled.

1005101212

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

5. Starts as a parenchymal disease and progresses into non-specific bronchitis (viral, sarcoid).

6. Chronic bronchtis, which mimics "pure emphysema". These are the patients with very discreet, but tenacious, expectoration. Still, this sputum has all the characteristics of inflammatory and/or infectious bronchitis. Because of it, there are few, if any, rhonchi or wheezing and, instead, silent areas and marked shortness of breath are present.

2. Sputum Studies:

A large experience has been accumulated in our laboratory. Evaluation of 4,000 sputum specimens in patients with "chronic bronchitis" has shown that the results are of statistical significance as indicators of early changes in the level of bronchial inflammation, and these determinations appear to be more sensitive than the clinical evaluation of the use of ancillary laboratory parameters like WBC and changes in the gross physical characteristics of sputum. Our special sputum studies acquired particular significance when analyzed in terms of follow-up changes for each individual. The clinical subgroups of bronchitis that were delineated were the "stable" patient without evidence of clinically acute bronchial inflammation (CB-S), the same type patient during the first few days of a clinically acute inflammatory episode (CB-AI). The differences that were noted between these groups were:

a) The blood polymorphonuclear neutrophils (PMN) alkaline phosphatase activity was significantly elevated in the BA-AI group vs. both other groups, although there was no discernable increase in WBC's or shifts-to-the-left. It is interesting that this response was generally lower than what has been observed in "normal" individuals with acute bacterial infection.

b) There is a significant increase in the mean number of PMN's excreted per day in the sputum between both the CB-S (156×10^6) and CB-P (320×10^6) groups and the CB-AI group (847×10^6).

c) There is a similarly significant increase in the number of histiocytes excreted per day in the sputum, the values being respectively: CB-S (3.5×10^6), CB-P (3.8×10^6) and CB-AI (12.5×10^6).

d) There is a significant increase in the number of bronchial epithelial cells excreted per day between the CB-S group (18.1×10^6) and the CB-AI group (64.9×10^6).

e) In the individual patient, these changes were often noted to begin before a clinically apparent acute inflammatory episode occurred. In addition, a further increase in histiocytes often occurred concurrently with the onset of clinical improvement.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

f) Significantly more of the sputum specimens demonstrated alkaline phosphatase activity in the histiocytes and bronchial epithelial cells in the CB-P group as compared to the CB-S group.

In a published study of 21 patients with chronic bronchitis, it was shown that during acute inflammatory processes, the excretion of all sputum cells was considerably increased, made up principally of neutrophil type, and certain differences in the type of epithelial cells excreted also occurred (Am. Rev. Resp. Dis., 85:635, 1962).

Methods for evaluating some of the physical characteristics of the sputum have been developed in our laboratory. A simple method for assessing the stickiness or "adhesiveness" and the thickness or "viscosity" has been tested in over 1,500 specimens. This involves the measurement of the time it takes the leading edge of an aliquot of sputum to reach successive distances in a 12 mm. tube on an inclined plane. When the time-distance relationships are plotted on semi-log paper, one can delineate a slope which seems to relate to "adhesiveness" and a second slope which seems to relate to "viscosity". Correlation of the mathematical expressions of these curves with pertinent symptoms of the patients at the time they raised the sputum show clear relationships of the measurement to symptoms referrible to sputum characteristics. We have been evaluating all of the study patients, utilizing these methods, but had not previously described the procedure.

The dry weight of the sputum samples is also being determined by drying aliquots of homogenized samples at 37°C. We are also recording the water loss rates on these specimens by periodic weighings during the drying process.

Over the past year, we have been working on methods to prepare sputum for protein separation by electrophoresis. By a sequence of ultrasonics and various precipitation methods, we have been able to prepare a clear fluid containing the dissolved sputum proteins. Electrophoresis of this material has resulted in rather clean separation of proteins. Although we still have to identify the proteins - for which purpose we plan to do concomitant serum protein electrophoresis and utilize immunologic techniques - it appears that we can begin to survey the study patient, using these techniques.

3. Laminographic Studies:

We have studied more than 80 patients by this technique. It has proven to be of extreme help in localizing even comparatively minor degrees of pulmonary vascular abnormalities in the presence of established chronic pulmonary emphysema. The pathological changes observed were: reduction in number of secondary branching; abnormal regional blood vessels crowding; signs of increased vascular resistance unequally distributed throughout the parenchyma and finally, massive disappearance of the peripheral circulation (Am. J. Roent., Rad. Ther. & Muc. Med., 88:1043, 1962).

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

At the same time, we became aware of 2 facts: a) that in far advanced emphysema, the laminography could often detect gross abnormalities in the configuration of the bronchi supplying parenchymal areas where vascular derangement was detected and b) the need for adding a direct radiological approach, e.g., bronchography to clarify this relationship. Furthermore, it became obvious that laminography was a good tool only at the stages of advanced established emphysema, but not for the earlier changes or in the presence of pure chronic bronchitis. Bronchography performed in many of these patients has considerably increased the accuracy of detecting the relative participation of bronchial versus parenchymal involvement. Still, some patients with definite clinical and sputum manifestations of bronchitis may go undefected by bronchography. A major problem is the development of better techniques for this visualization, particularly the need for attaining maximal bronchial cleansing prior to bronchography and by using cinebronchographic sequences. Finally, and more important, a continuous attempt is being made by our group to learn from the findings in laminography and bronchography how to improve the plain chest X-ray reading (Radiologic Contributions to the Nosology of Obstructive Pulmonary Syndrome. Accepted for publication by Dis. Chest in 1965).

4. Community Chronic Lung Disease Study:

For the past year, we have been participating in a program designed to study methods for the postgraduate education of private physicians in the current trend in the diagnosis and treatment of chronic pulmonary disease. This has been carried out in cooperation with the Norfolk County TB and Health Association. Through this program, we have had the opportunity to apply many of the clinical and laboratory techniques described for the present study. This experience has been extremely valuable in the planning of this current research proposal.

5. Chronic Pulmonary Disease - Peptic Ulcer Interrelationships:

A mass of information was accumulated in the study of the relationship of chronic pulmonary disease to peptic ulcer disease and smoking habits. The data was previously submitted (June 12, 1963) to Dr. Hockett on 60 patients with chronic pulmonary disease (CPD) and 15 matched controls with chronic non-pulmonary disease (CD). The results indicated that although 52 per cent of the CPD group has reasonable evidence for peptic ulcer disease, 40 per cent of the CD group also had this finding. The non-smokers in the CPD group had a 44 per cent incidence. Also of interest is the finding that 18 per cent of the CPD group never smoked. It would appear that there are many etiologic factors to be considered in the understanding of chronic pulmonary disease. The occurrence of peptic ulcer disease seems to be related to factors of chronic illness in general, rather than specifically to chronic lung disease or smoking.

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Source: https://www.industrydocuments.ucsf.edu/docs/mtwn0000

B. Pertinent Literature References

In the past 5 years, there has been a flood of increasing interest in the field, but none of the proposed clinical classifications of the chronic bronchitis syndrome has found widespread acceptance. In view of this, the use of terminology such as "chronic non-specific obstructive pulmonary disease" (Ciba Guest Symposium, Thorax, 14:286, 1959) is wise at present.

A most recent attempt at a working classification has been put forward by Dr. P. Hugh-Jones (Boston City Hospital Lecture, April, 1963) who uses terms such as "non-paroxysmal asthma" (wheezing, asthmatic bronchitis); "simple bronchitis" (smoker's cough?); "infected bronchitis" (simple, plus infection?); "bronchitis and emphysema"; and "pure emphysema" (shortness of breath only). Within this working classification, he found that 2 clinico-pathological correlations seemed most important: "Pure infected bronchitis" usually exhibits centrilobular destruction, whereas "pure emphysema" usually is characterized by panacinar destruction.

This concept also has a physiological counterpart. In the bronchitis group, the obstructive element leads to some abnormalities in intrapulmonary gas exchange, whereas in emphysema the shortness of breath is mostly the result of abnormal mechanics of breathing.

Bronchography in recent years has shown its potentialities as a descriptive method in the study of chronic bronchitis.

Early bronchographic reports by Fisher, F.K. (In Roentgen Diagnosis by Scintz et al., English Editor, J.T. Case, London, 1953) describe generalized bronchial narrowing in bronchitis and in asthma, Simon (In Recent Trends in Chronic Bronchitis, N.C. Osward, Editor, Lloyd-Lude, London, 1958) describes different bronchographic patterns in chronic bronchitis such as peripheral pool, local dilatations of proximal bronchi, beading, peripheral lack of filling, etc. The description is quite extensive, but again, there is no clinical indentification of the entities responsible for these various changes, and they are all grouped within the chronic "bronchitis group".

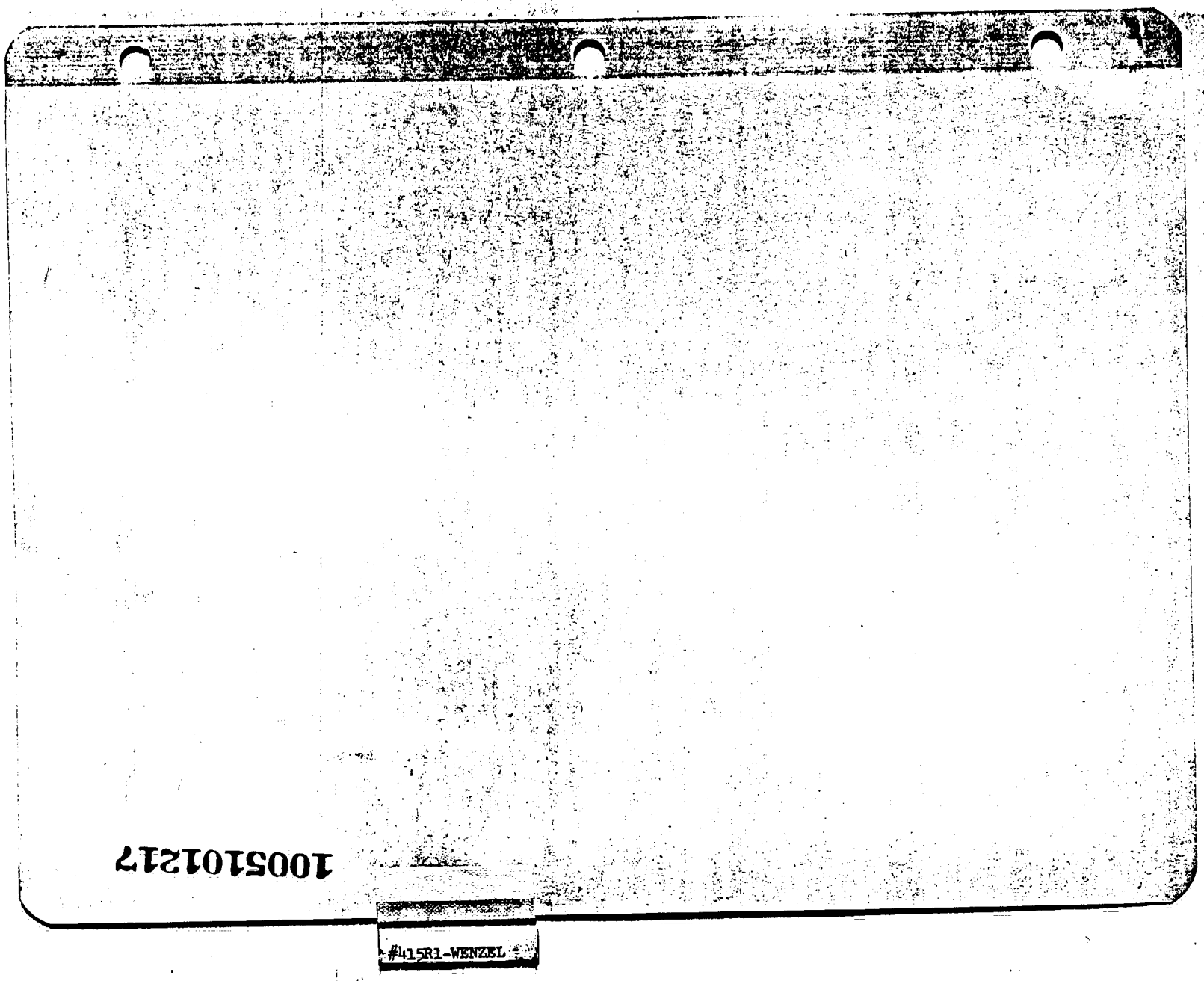
Recently, a new important diagnostic step was added when Leopold and Seal (Thorax, 16:70, 1961) described the bronchographic appearance of centrilobular emphysema which was subsequently confirmed at autopsy. This pathological diagnosis was hitherto impossible to make in vivo.

Signature Maria S. Seal M.D.
Director of Project

Richard V. Thompson
Business Officer of the Institution
Accounting and Budget Officer

1005101216

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000



Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

CONFIDENTIAL

February 14, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chairman, Dr. Bing and Dr. Little.

FROM: Robert C. Hockett

SUBJECT: Renewal application of Duane G. Wenzel, Ph.D. - #415R1.

We enclose herewith a renewal application, accompanied by a progress report, from Dr. Duane G. Wenzel of the University of Kansas.

The initial grant for this project was for one year without further commitment. The research plan for an additional year represents an extension of the work completed during the first year.

This application is being sent at present only to members of the committee, but we plan to include it in the agenda for the March meeting.

R.C.H.

RCH:ek

encls.

Personal Opinion: Dr. Wenzel seems now to be making pretty good progress and has obtained some interesting results, which probably warrants another year's support for the program. (A site visit was made on January 28.)

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#415R1
Activated: 1/1/65
Cf. #155
7/1/57 - 9/1/59
Cf. #345
9/1/62 - 2/28/64

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Little

Renewal
Application For Research Grant

Date: February 1, 1966

1. Name of Investigator: DUANE G. WENZEL, PH.D.

2. Title: Professor of Pharmacology

3. Institution & Address:
The University of Kansas
School of Pharmacy
Lawrence, Kansas 66044

4. Project or Subject:

ANTIHYPERTENSIVE ACTIVITY OF NICOTINE

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Introduction: Work from our laboratories has been reported which demonstrates that the chronic oral administration of nicotine to rats first elevates the systolic blood pressure and then reduces it to the original level or below.[1] Further, renal hypertension was reduced to normal by this treatment. Since the above study was reported, the project supported by the Tobacco Research Council has made the following general findings to date: (more detailed evidence in attached progress report)

(1) Increasing the dose of nicotine from 1.14 or 2.28 mg/kg/day to 4.56 mg/kg/day eliminates the initial pressor response.

(2) This larger dose administered concurrently with the production of renal hypertension permits the development of hypertension, although the pressor response peaks and falls faster than when a lower dose of nicotine was employed in the previous study (3 weeks vs. 15 weeks).

(3) Beginning administration of the larger dose of nicotine to rats with fully developed renal hypertension produces a rapid fall in blood pressure (30 mm Hg in three weeks).

1. Wenzel, D. G., Wattanapongsiri, A. and Vedral, D., Nicotine and Renal Hypertension in the Rat, J. Pharmacol., 145, 315 (1964).

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

5. Detailed Plan of Procedure (continued)

(4) Rats treated with 4.56 mg/kg/day of nicotine for 14 weeks demonstrate increased sensitivity to the pressor effect of norepinephrine; a reduced response to tyramine; a slight but significant sensitization to vasopressin; and no change in the response to angiotensin.

On the basis of the above results and the known effects of nicotine on catecholamine stores, the following tentative hypothesis is offered as an explanation: The pressor effect of nicotine is related in large measure to the release of norepinephrine stores while the depressor effect results from the depletion of these stores.

This hypothesis will be directly and indirectly tested by a combination of two methods: (1) measurement of the contractile response of norepinephrine-dependant isolated smooth muscle from rats chronically treated with nicotine, and (2) measurement of epinephrine and norepinephrine stores in this muscle as well as in other tissues from the same animals.

Basis for Use of Vas Referens: Initial investigations have been underway since the summer of 1965 to determine the suitability of the rat hypogastric nerve-vas deferens preparation for the study of the chronic effects of nicotine. An improved apparatus has been designed to measure the isotonic contractions of the vas deferens when stimulated alternately through the muscle itself and through the hypogastric nerve. Details of the effects of varying stimulus parameters are still under study.

The isolated hypogastric-vas deferens preparation is being employed because of its unique characteristics. It is one of the few satisfactory isolated nerve-smooth muscle preparations currently known having autonomic ganglia, post-ganglionic adrenergic fibers, and pre- and post-ganglionic cholinergic fibers. Further, the effects of chronic nicotine treatment at these sites may be determined through the use of different electrical stimulus parameters and the use of pharmacological agents added in vitro ie. Low frequencies (5-10 cps) through the hypogastric nerve release acetylcholine at the end organ while high frequencies (25-50 cps) release norepinephrine. High frequencies, applied directly to the muscle, stimulate postganglionic adrenergic fibers while low frequencies of long duration directly stimulate the smooth muscle cell.

Experimental Design: Two separate groups of male Sprague-Dawley rats will be administered 1.14 or 4.46 mg/kg/day of nicotine in the drinking water. Eight rats will be sacrificed from each group on days 1, 7, 21, 42 and 84. A similar untreated control group will be used with eight animals for each period of testing. The mean contractile force of the electrically-stimulated vas deferens-hypogastric nerve preparations will be determined at frequencies of 1, 5, 15, 30 and 50 cps applied alternately to nerve and directly to the muscle. Pulse duration is to be 2 milliseconds, period of stimulation 5 seconds, and stimulus amplitude supramaximal (20 volts nerve and 60 volts muscle). Once the frequency-response series is completed for both nerve and muscle on a given preparation, it will be treated in vitro with atropine (1×10^{-6}) with responses determined at 5 and 50 cycles, and next with phentolamine (0.1×10^{-6}) using the same test procedure.

Further evaluation of the effects of chronic nicotine upon the vas deferens will depend upon initial results. It is anticipated that it will be both desirable and necessary to essentially duplicate the above experiment with the substitution of acetylcholine or norepinephrine for electrical stimulation.

Part II. Concurrently with the above procedure, specific organs will be isolated from each test animal and will be analyzed for their epinephrine and norepinephrine content by spectrofluorometry. The organs will include: the remaining vas deferens, both adrenals, heart, and aorta.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$ 8,280
b.	Expendable Supplies	1,200
c.	Permanent Equipment	750
d.	Overhead (15% of a, b, e)	1,452
e.	Other	200
	Total	$11,882

7. Anticipated Duration of Work: The study, as outlined, should take about one year. (See 10)

8. Facilities and Staff Available: The School of Pharmacy has six pharmacology research laboratories. One or more of these would be devoted exclusively to continuing the research relative to the effect of nicotine on the blood pressure. A modern, fully-staffed animal room is used for such research programs. A Science Library holding over 60,000 volumes occupies one floor of our building.

The School of Pharmacy has four pharmacologists on its staff as well as four medicinal chemists and specialists in other areas who may serve as consultants.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply): (From 7)

If the results of this year's study corroborate the evidence that nicotine is acting to produce a chemical sympathectomy, further work will be desirable in order to (1) make a comparison of the blood pressure effects and side-effects of chronic nicotine and syrosingopine treatments; (2) determine the effect of chronic nicotine upon rats having genetic and salt-DOCA hypertension; (3) determine the systolic and diastolic b.p. response of chronic nicotine-treated rats subjected to drugs (eg. pressor agents) and treatments (eg. carotid occlusion) via arterial cannulation.

Signature Duane G. Wengel
Director of Project

William J. Argersinger, Jr.
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CONFIDENTIAL

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

February 14, 1966

MEMORANDUM

TO: The committee comprising Dr. Bing, Chm., Dr. Cattell and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application of Domingo M. Aviado, M.D. - No. 436R2.

We enclose herewith a renewal application from Dr. Domingo M. Aviado of the University of Pennsylvania School of Medicine, accompanied by a progress report.

This represents the final year of an original three-year request. The anticipated duration of work is for one year as originally planned. Last year the applicant requested the same amount as is applied for in the present application, but the Board approved the proposal at the same rate as the previous year -- namely $13,418.00. The research program has been expanded and the present request is for $19,250.00.

The progress report consists of four reprints (mailed to the Scientific Advisory Board on September 30, 1965) and the three manuscripts enclosed.(List at botton

This application is being sent at present only to members of the committee, but we plan to include it in the agenda for the March meeting.

R. C. H.

1005101223

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

PHYSIOLOGY OF THE RESPIRATORY SYSTEM

No. 436R2

Activated: 7/1/64
Renewed: 4/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Bing, Chm.
Dr. Cattell
Dr. Jacobson

Application for Renewal of Research Grant

Date: January 17, 1966

1. Name of Investigator: Domingo M. Aviado, M.D.

2. Title: Professor of Pharmacology

3. Institution & Address: University of Pennsylvania School of Medicine
Department of Pharmacology
36th & Hamilton Walk
Philadelphia, Pennsylvania 19104

4. Project or Subject: Effects of nicotine and cigarette smoking on neurogenic mechanisms in the lung.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Our progress report which is being submitted with this proposal consists of four published articles on the bronchopulmonary[1-4] effects of tobacco, and of three manuscripts (submitted for publication) on the cardiopulmonary[5-7] effects of tobacco. These articles deal on work during the past two years and have served the purpose of establishing the existence of neurogenic and humoral mechanisms in the lung which are activated by cigarette smoking and elicit changes in bronchomotor tone, bronchial blood flow, pulmonary blood pressure, cardiac output, and coronary vascular resistance. The major theme of the experiments proposed for the third year is on the effects of tobacco on respiratory control, blood-gas exchange and pulmonary compliance and resistance to breathing. The details of the poposed experiments are as follows:

a. Respiratory control. The participation of the medullary centers and chemoreceptors to the respiratory effects of tobacco will be investigated in the anesthetized dog. Since most of previous experiments are on nicotine, the planned experiments will be on the effects of blood exposed to cigarette smoke directly on the respiratory center and on the chemoreceptors in the carotid, aortic and pulmonary bodies. The perfusion and cross circulation technics will be applied to these areas to assess the relative importance of centra and receptor mechanisms.

b. Blood gas exchange. The tension of oxygen and carbon dioxide of blood from the lung exposed to cigarette smoke will be measured. The apparatus for direct polarographic methods has been assembled and ready to apply to blood samples collected from the anesthetized dog. Special attention will be paid to the participation of arteriovenous communications and bronchopulmonary blood vessels in contributing to the venous admixture in the pulmonary veins.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

c. Pulmonary compliance and resistance. Dogs and smaller animals will be exposed daily to cigarette smoke. Pulmonary compliance and airway resistance will be measured daily. After the appearance of significant alterations in lung function, the animal will be anesthetized and measurements taken with the open chest. Finally the lung will be excised and artifically perfused for comparison with the lung excised from the animal not previously exposed to cigarette smoke. The tissue will also be examined histologically for lesions in the pulmonary blood vessels and bronchibles.

The senior investigator is hopeful that after the information from the proposed experiments become available, plans for a second three-year project devoted to the relationship of tobacco to the pathogenesis of chronic pulmonary emphysema, bronchial spasm and bronchial neoplasm can be formulated.

References cited above and submitted as progress report no. 2.

1. Aviado, D. M. and Samanek, M.: Bronchopulmonary Effects of Tobacco and Related Substances: I. Bronchoconstriction and Bronchodilatation: Influence of Lung Denervation. Arch Environ Health 11: 141-151, 1965.

2. Samanek, M., and Aviado, D. M.: Bronchopulmonary Effects of Tobacco and Related Substances: II. Bronchial Arterial Injections of Nicotine and Histamine. Arch Environ Health 11: 152-160, 1965

3. Samanek, M., Aviado, D. M. and Peskin, G. W.: Bronchopulmonary Effects of Tobacco and Related Substances: III. Axon Reflexes Elicited from the Visceral Pleura. Arch Environ Health 11: 160-166, 1965.

4. Samanek, M. and Aviado, D. M.: Bronchopulmonary Effects of Tobacco and Related Substances: IV. Bronchial Vascular and Bronchomotor Responses; Their Suggested Defense Function. Arch Environ Health 11: 167-176, 1965.

5. Aviado, D. M., Samanek, M. and Folle, L. E.: Cardiopulmonary Effects of Tobacco and Related Substances: I. The Release of Histamine During Inhalation of Cigarette Smoke and Anoxemia in the Heart-Lung Preparation. To be submitted to the Arch Environ Health.

6. Folle, L. E., Samanek, M. and Aviado, D. M.: Cardiopulmonary Effects of Tobacco and Related Substances: II. Coronary Vascular Effects of Cigarette Smoke and Nicotine. To be submitted to the Arch Environ Health.

7. Samanek, M. and Aviado, D. M.: Cardiopulmonary Effects of Tobacco and Related Substances: III. Pulmonary Vascular Effects of Cigarette Smoke and Nicotine. To be submitted to the Arch Environ Health.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Budget Plan:

	Salaries	9,000
a.	Salaries (9% employee benefits)	
b.	Expendable Supplies	4,600
c.	Other Expenses	2,000
d.	Permanent Equipment	500
e.	Overhead (15% of a, b, c)	2,340
	Total	19,250

7. Anticipated Duration of Work

One year.

8. Facilities and Staff Availabk: We have increased our original yearly budget by 50% chiefly because we would like to embark on this project more extensively than we have for the past two years. The requested amount for salaries will be for a postdoctorate fellow ($5, 000) and a technician ($4, 000) who will spend full time on this project, under the pervision of the Director of the project. The expendable supplies will largely be for the cost of animals (dogs, rabbits, rats and guinea pigs). The additional pieces of ermanent equipment will include microtone and microscope for examining lung tissue section.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other, sources of support):

This request is not being covered by other sources of support. The undersigned has a grant of $49, 000 annually from the Department of the Army for the project entitled "Pharmacolbgy of New Antimalarials."

Signature

irector of Project

[redacted] Lu L. Terry, M.D.
Vice President for Medica Affairs.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CONFIDENTIAL

MEMORANDUM

TO: The committee comprising Dr. Bing, Chm., Dr. Jacobson and Dr. CAttell.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Ulrich Schaeppi, M.D. - No. 447R1.

We enclose herewith a renewal application, with an appendix reporting progress, and one reprint from Dr. Ulrich Schaeppi of the Worcester Foundation for Experimental Biology.

This application represents the second year of a two-year program with committed support. However, it should be noted that a new program has been added to the renewal plan and that the latter is projected for two years. This addition increases the budget over that originally contemplated for the second year.

The proposal is being sent at present only to members of the committee, but we plan to include it in the agenda for the March meeting.

R. C. H.

Personal Opinion: Progress has been excellent and I favor continued support. The new program is indeed more directly relevant to our interests than the original one.

C. H.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#447R1
Activated: 6/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Bing, Chm.
Dr. Jacobson
Dr. Cattell

Renewal
Application for Research Grant

Date: February 4, 1966

1. Name of Investigator: ULRICH SCHAEPPI, M.D.

2. Title: Staff Scientist

3. Institution & Address: WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
222 Maple Avenue
Shrewsbury, Massachusetts 01545

4. Project or Subject: (A) Non-ganglionic Parasympathomimetic Action of Nicotine upon the Iris Sphincter.
(B) Changes in the Level of Vigilance Elicited by Administration of Nicotine to the Lower Brain Stem.

5. Detailed Plan of Procedure (Use additional pages if more space is required)

Summary

(A) Non-ganglionic Parasympathomimetic Action of Nicotine upon the Iris Sphincter.

We have observed that nicotine produces contraction of isolated mamalian iris sphincters. This effect occurs by a direct action of nicotine upon postganglionic parasympathetic nerve endings and leads to the release of cholinergic transmitter (and the latter induces then sphincter contraction). This interpretation is supported by the following observations:

1) the nicotine induced sphincter contraction shows the characteristic of a mechanism which is mediated by release of cholinergic transmitter (Schaeppi and Koella, 1964; Schaeppi and Dodd, 1965);

2) isolated iris sphincters react to physiological and pharmacological procedures like ganglion-free preparations (Schaeppi, 1966);

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

3) nicotine fails to produce contraction of chronically parasympathetically denervated irides (Schaeppi and Dodd, 1965; Schaeppi, 1966).

In future work we wish to extend our observations on postganglionic parasympathomimetic effects of nicotine upon irides which will be investigated in vivo.

The iris sphincter serves as a model to demonstrate that nicotine stimulates the peripheral parasympathetic neuron not only at ganglionic sites (Magnus, 1905) but also at postganglionic nerve endings. This new mechanism of action of nicotine will help to understand (a) the action of nicotine upon other peripheral parasympathetic neurons (for instance those innervating coronary arteries), and (b) it will help to understand the nicotine effect upon brain systems containing cholinergic neurons.

For further information see appendix A.

Summary

(B) Changes in the Level of Vigilance Elicited by Administration of Nicotine to the Lower Brain Stem.

We have recently observed that nicotine injected into the 4th ventricle of unanesthetized paralyzed cats which are kept under local anesthesia produces a marked change in the level of vigilance which consists of a phase of arousal followed by a phase of reduced vigilance.

In the future we wish to further investigate the effects of the localized administration of nicotine to the brain stem. In particular we wish to study the effects of the injection of nicotine

1) into the blood supply to the lower brain stem;

2) into the cisterna magna;

3) into the 3rd ventricle;

4) also, we wish to investigate the effects of nicotine administration to localized areas on the floor of the 4th ventricle.

Injection of very small doses of nicotine into the 4th ventricle elicits marked changes in the level of vigilance of unanesthetized paralyzed cats. To what extent these nicotine effects play a role upon the production of psychic changes occurring in response to systemic administration of nicotine or in response to nicotine uptake during tobacco smoking remains to be seen.

For further information see appendix B.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

a. Salaries	Schaeppi=50%; Dodd=100%	$ 12,040
b. Expendable Supplies		3,000
c. Other Expenses		00
d. Permanent Equipment	** (see below)	2,530
e. Overhead (15% of a. b, 4		3 0
	Total	$ 21,400

7. Anticipated Duration of Work Two years.

8. Facilities and Staff Available: I have two laboratories at my disposal, Furthermore, a complete set-up for the investigation of pupillary changes. It consists of Grass 2-channel polygraph, 1 S-4 Grass stimulator, a Heath Kit Oscilloscope monitoring electrical stimulation. Sundry equipment as balances, etc., are likewise at my disposal. To ihvestigate central effects of nicotine an operation table with a stereotaxic instrument is also available. Miss M. Dodd is full time technical assistant.

9. Additional Requirements: The $3,530.00 listed for aquisition of permanent equipment will be spent to buy the equipment listed on the attached page.

10. Additional Informalion (Including relation of work to other projects andlother sources of support), 50% of my time will be used to investigate central mechanisms which are involved in the control of the cat's level of vigilance. This work is supported by a National Institute of Health grant to Dr. W. P. Koella.

**ermanent Equipment: (Grass Polygraph with accessories for recording of EG, Blood pressure, tension. of nictitating membrane, etc.)

channel console		$ 550.00
r er amp if ers	4 x 180.00	720.00
oscillographs	4 x 200.00	800.00
ignaL marker		120.00
low level DC 5P1	2 x 295.00	590.00
low level AC	3 x 125.00	375.00
Grass force displacement transducer	FT 03	125.00
1 Statham blood pressure strain gauge	P 23 AC	250.00

Director of Projed Ulrich Schaeppi, M, D.

Business Officer of the Institution

R: REDACTED MATERIAL

CURRICULUM VITAE

Ulrich H. Schaeppi, M. D.

REDACTED

CITIZENSHIP: American citizen

REDACTED CHILDREN: Four

EDUCATION: University of Zurich and Berne - M. D., 1951

EXPERIENCE: Resident in internal medicine, Kantonsspital Aarau, 1952-1955. Director: Professor Dr. A. Alder.
Resident-Institute of Pharmacology, University of Zurich, 1956-1957. Director: Professor Dr. P. Waser.
Pharmacologist, J. R. Geigy, A. G. Basel, 1957-1960. Director: Professor Dr. H. Wirz.
Neuropharmacologist, The Worcester Foundation for Experimental Biology, Shrewsbury, Mass., 1960 -
Director: Dr. W. P. Koella.

MILITARY SERVICE: Swiss Army (Rank: First Lieutenant).

PUBLICATIONS: Approximately 25 papers in scientific journals.

REDACTED

SPEAKING ENGAGEMENTS: Invited speaker at the International meeting sponsored by the New York Academy of Sciences April 7-9, 1966 in New York, N. Y. "The effects of nicotine and smoking on the central nervous system."

FINANCIAL SUPPORT: My work is supported in part by a personal research grant from The Council for Tobacco Research, U.S.A., and in part by a research grant from the National Institute of Health to Dr. W. P. Koella.

January, 1966

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

(A) Non-ganglionic Parasympathomimetic Action of Nicotine upon the Iris Sphincter.

Present status of work:

The question whether nicotine stimulates autonomic effectors which are innervated by parasympathetic nerve fibers by an action upon ganglion cells only or whether it elicits also effects at postganglionic sites was not well understood in the past.

We have recently observed that nicotine produces contraction of the isolated iris sphincter of the pig (Schaeppi and Koella, 1964a). A grant from the Tobacco Research Council of U.S.A. for 1965-66 made it possible to further investigate this nicotine effect. In particular we were able to demonstrate that the pig iris reacts as if its parasympathetic nerve supply does not contain ganglion cells. This conclusion is borne out by observations that iris sphincters from eyes which were subjected to ciliary ganglionectomy two weeks prior to the in vitro investigation show the characteristic symptoms of chronic parasympathetic denervation. These symptoms consist of supersensitivity to small doses of acetylcholine and of neural inexcitability (Schaeppi, 1966). The observation that the parasympathetic nerve supply reacts as if it does not contain ganglion cells indicates then that the nicotine induced contraction of the isolated pig iris sphincter has to be attributed to a non-ganglionic site of action of nicotine. The further observation that nicotine fails to produce contraction of chronically parasympathetically denervated iris sphincters indicates that nicotine does not elicit contraction by a direct action upon the muscle fiber. It rather indicates that the nicotine induced contraction of the normal iris sphincter is due to the release of cholinergic transmitter from nerve endings or from structures depending upon an intact parasympathetic nerve supply. A detailed report on the postganglionic stimulating and blocking effects which nicotine exerts upon parasympathetic nerve endings in the pig iris sphincter has been published (Schaeppi and Dodd, 1965; Schaeppi, 1966), see also Figure A-1. The observation that nicotine produces postganglionic effects resembling those of peripheral parasympathetic stimulation can be compared with observations by Burn et al. (1959) who found that nicotine produces postganglionic effects resembling those of peripheral sympathetic stimulation.

Non-ganglionic effects of nicotine, similar to those observed in the isolated pig iris sphincter, can be found in isolated intact cat irides. A report on these observations is at present prepared for publication (Schaeppi and Koella, 1966) (see also Figure A-2).

Future work:

We wish to continue our work and to extend our observations on nicotine effects upon irides in vivo. Atkinson et al. (1965) recently have studied the effects of the intracarotid injection of nicotine in anesthetized cats. They observed that nicotine produced marked sympathomimetic effects but they failed to observe a postganglionic parasympathomimetic action. It must be expected that catecholamines which are released from the walls of the blood vessels to the iris may have contributed

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to the sympathomimetic action of nicotine and that strong sympathomimetic effects may then completely have masked parasympathomimetic effects of this compound.

In our future work we will investigate the effects of nicotine upon in vivo irides and we will inject nicotine into the anterior eye chamber of three different cat preparations.

Detailed plan of procedure:

Cats are operated under ether anesthesia. The trachea is cannulated and a polyethylene cannula is tied into the femoral vein. The size of both pupils is continuously monitored by means of a set of two pupillometers which are at present developed in our laboratories. Nicotine is injected locally into the anterior eye chamber by means of the following technique. A 22 gauge needle is inserted from the limbus corneae in a sense that the tip of the needle is located in the anterior eye chamber, in the center of the pupil. Thereafter, the needle is rigidly fastened and serves as a guide for a 28 gauge needle containing nicotine solution. Nicotine is dissolved in Tyrode solution and it is injected in 0.025 ml volumes. To maintain constant pressure within the eye ball a third needle is inserted into the anterior eye chamber and connected to a Tyrode reservoir with constant pressure. The nicotine effect is investigated in three different cat preparations:

1) Cats in deep Nembutal anesthesia in which the oculomotor nerve was transected within the skull are used to study the action of nicotine upon the mydriatic pupil. This preparation serves thus to demonstrate the miotic effects of nicotine.

2) Cats subjected to postcollicular pretrigeminal decerebration will be used as an in vivo preparation with firmly closed pupil. This preparation serves to demonstrate mydriatic effects of nicotine.

3) Cats maintained under deep pentobarbital anesthesia will be used to investigate the action of nicotine upon irides with intermediate pupillary size. These preparations will be used to investigate the biphasic response of nicotine which consists in miosis followed by mydriasis.

We will use only observations from cats in which nicotine failed to change the pupillary size of the non-treated control eye.

We also wish to investigate whether nicotine-induced miosis is mediated by a cholinergic mechanism. We will therefore investigate the effects of nicotine upon irides treated with atropine or inhibitors of cholinesterase and upon irides which were subjected to chronic parasympathetic denervation.

Significance:

Nicotine was found to produce stimulation of postganglionic parasympathetic nerve endings. This observation adds a new perspective to the mode of action of nicotine upon the cholinergic neuron, and contributes to a better understanding of the complex action of this compound. Whether this action has any bearing on experiments involving systemic administration of nicotine and particularly on long-term effects of smoking, remains to be seen.

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ory fluctuations of the sympathomimetic response of the cat iris to nicotine.
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on-ganglionic parasympathomimetic action of nicotine upon the isolated cat
iris.
(In Preparation for Publication).

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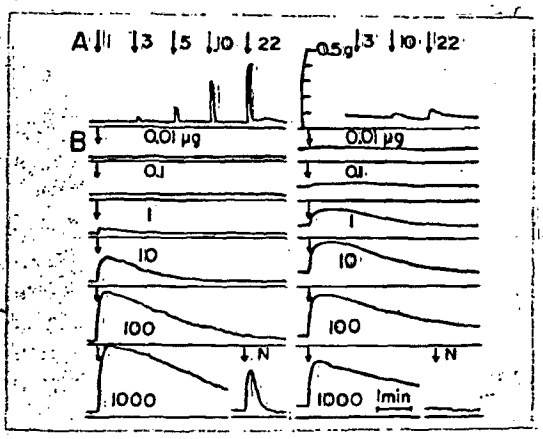
Appendix

Figure A-1: Tension changes of normal pig iris sphincter (column at left) and of contralateral chronically parasympathetically denervated iris sphincter (column at right) in response to electrical stimulation, acetylcholine and nicotine (N).



A: arrows indicate beginning of 5-sec periods of electrical stimulation with pulses of 1 msec duration at 15 pulses/sec and volts as indicated. B: arrows indicate administration of acetylcholine in doses as indicated except arrow marked N, which shows administration of 50 mg nicotine. Both preparations investigated at interhook distance of 9 mm. Both preparations same force calibration (grams). Note: Nicotine produces contraction of the normal sphincter but fails to produce contraction of the chronically parasympathetically denervated preparation.

From: Schaeppi, U., Am. J. Physiol. 210: 91-94, 1966.

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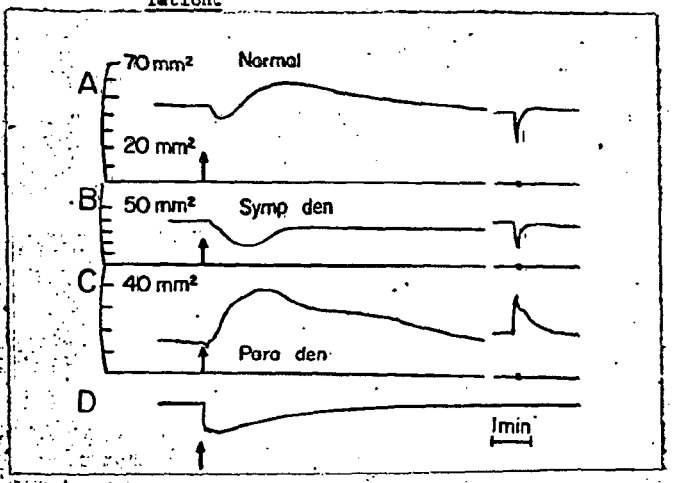
Figure A-2: Changes in pupillary size of isolated intact cat irides in response to administration of nicotine and in response to electrical stimulation.



Ordinate: pupillary size in mm^2. Abszissa: time. Arrows: A, B, and C injection of nicotine 10 mg. Markers: electrical stimulation with 5 sec trains of pulses of 2 msec pulse width, 3 pps at 20 volts. A: normal, B: chronically sympathetically denervated and C: chronically parasympathetically denervated intact cat iris. D: changes in transparency (arbitrary units) after a single injection of india ink. Iris replaced by metal plate with circular opening of 50 mm^2. Arrow injection of india ink. Flow rate of Tyrode 20 ml/min. Note: In normal irides nicotine produces closure followed by opening. After elimination of the parasympathetic nerve supply to the iris (chronically parasympathetically denervated iris) nicotine produces only opening. In the chronically sympathetically denervated iris nicotine produces only miosis.

From: Schaeppi, U., Dennison, B.L., and Dodd, M., "Non-ganglionic parasympathomimetic action of nicotine upon the isolated cat iris". (In Preparation for Publication)

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

(B) hanges in the Level of Vigilance Elicited by Administration of Nicotine to the Lower Brain Stem.

ntroduction:

everal investigators have demonstrated that electrical or chemical timulation of the cat's lower brain stem exerts a marked effect pon the animal's level of vigilance. In enc phale isolé osts,, electrical stimulation of the medulla elicits, in dependence upon the timulus parameter and upon the site of atimulation, either symptoms of rousal or symptoms associated with a decrease. in the level of vigilance (Magnes et al., 1961). The elimination of the lower brain stem y surgical procedures (Batini et al., 1959), or the inhibition by ocal treatment with anesthetics (Magni et al., 1959; Bonvallet and loch, 1961) induces a shift towards arousal. This arousal effect is ue to the inactivation of medullary centers which exert a depressor ffect upon more rostrally located brain structures. Strychnine injected into the cat's fourth ventricle produces generalized arousal haeppi and Koella 1966) and this effect must be attributed to the ctivation of excitatory structures which are located in the lower rain stem. Serotonin injected into the cat's fourth ventricle induces a protracted decrease in the cat's level of vigilance and this effect was found to be due to the activation of chemoreceptors in the rea postrema which, induces the activation of medullary depressor enters (Koella et al., 1965).

icotine was repeatedly shown to have a direct action upon the lower rain stem of the cat. Medullary actions of nicotine consist in nesis (Laffanand Borison, 1957) and in changes in the rate and in mplitude of respiration (Nicholson and Sobin, 1938;, Mitchell et. l., 1963). It was therefore of interest to investigate medullary ffects of nicotine upon the cat's level of vigilance.

resent status of work:

Fifteen cats paralyzed with Flaxedil ere artificially respirated nd kept under local anesthesia., The level of vigilance was determined by recording cortical EEG, tension of the nictitating membrane, ize of pupil, blood pressure and ECG. Nicotine HC1 was dissolved in Tyrode, neutralized, and injected in volumes of 0,025 ml into the ourth ventricle. Nicotiine produced arousal in drowsy and sleeping ats. The arousal often was interrupted by short periods of EEG synhronization with decreased sympathetic activity. The phase of rousal usually was followed by a phase of reduced level of vigilance. The threshold dbse for these nicotine effects war about 1 µg. see Figure 8-1.) (Schaeppi, U.,,1966.)

tture work:

icotine administered to the medulla of cats affects respiration in dual way and this dual action has been shown to be caused by an ction upon at least two different medullary sites (Mitchell et al. 963). It is therefore very likely that the dual nicotine effect pon the cat's level of vigilance is also due to a multiple site of

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action upon the medulla. To further localize these sites we wish to continue our work with Flaxedil paralyzed cats and we wish:

1) to further investigate the action of nicotine injected into the cat's fourth ventricle;

2-3) to investigate the effects of nicotine injected into the cisterna magna and into the third ventricle;

4) to investigate the action of nicotine injected into the blood supply to the lower brain stem;

5) to investigate in cerebellectomized cats the effects of the local application of nicotine to various areas on the floor of the fourth ventricle;

6) to investigate the effects of nicotine administration into the fourth ventricle of unrestrained, free moving cats.

Detailed plan of procedure:

1) preparation and recording techniques:

Cats are tracheotomized under ether anesthesia and a polyvinyl cannula is tied into the femoral vein. The head is placed in a stereotaxic instrument and burr holes are drilled into the exposed skull (11 mm laterally from the sagittal suture and 12, 15 and 18 mm posterior from the coronal suture). Silver electrodes mounted on a lucite holder are inserted in the burr holes in a sense that the uninsulated tip is located on the dura. The lucite holder is fastened with screws to the skull. After termination of the surgical procedure the wound margins are infiltrated with 1% Xylocaine solution, the cats are maintained under artificial respiration and kept paralyzed with i.v. injection of Flaxedil 1 ml/hour. The electrocorticogram from the suprasylvian gyrus is recorded against an indifferent screw electrode on the frontal bone. The EEG from the medulla is monitored by means of stainless steel electrodes (Hess) which are mounted on a microdrive, and which are vertically inserted into the medulla. The EEG from the medulla is recorded from the area of the solitary nucleus against an indifferent screw electrode on the frontal bone. For electrical recording two 4 channel Grass polygraphs are used. The cortical EEG is routinely fed into an EEG Offner frequency analyzer whose output is recorded at 10 sec delay on the polygraph. The following indicators are also recorded on the polygraph: the blood pressure from the femoral artery by means of a Statham strain gauge, the tension of the nictitating membrane by means of a Grass force transducer and the size of the pupil by means of a pupillometer which was recently developed in our laboratories (Dennison, to be published).

2) a) For cannulation of the 4th ventricle a 22 gauge needle with short bevel is mounted on a microdrive. The cannula enters the skull through a burr hole in the crista occipitalis 3 mm in front of the eminentia occipitalis. The cannula is directed

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in the sagittal plane at an angle of from 60 to 70 degrees with the horizontal plane, and it is inserted until the tip reaches the fourth ventricle.

b) For cannulation of the cisterna magna the 22 gauge cannula is inserted horizontally through the membrana atlanto occipitalis. The needle perforates the membrane at the dorsal edge of the foramen magnum.

c) For cannulation of the third ventricle a 22 gauge cannula is inserted vertically through the parietal bone. It is oriented in the sagittal plane, enters the parietal bone through a burr hole in the sagittal suture, 12.5 mm anterior to the interauricular line, and it is inserted until it reaches the 3rd ventricle. To prevent nicotine injected into the 3rd ventricle from reaching pontine and medullary structures the liquor outflow from the 3rd ventricle is collected by means of a polyethylene tube which is inserted in the aqueduct of the midbrain. For insertion of this tube the cat is cerebellectomized and the tube is inserted under visual control.

d) To inject nicotine into the 4th ventricle of unanesthetized unrestrained cats we will use the technique of ventricular cannulation as reported by Feldberg and Sherwood (1953). We will use principally the same transcerebellar approach as described in (a) above.

e) For the application of nicotine to localized areas on the floor of the 4th ventricle the cerebellum is removed under local anesthesia. Cotton pellets of 2 mm diameter soaked with nicotine dissolved in Tyrode solution are applied to various areas on the floor of the 4th ventricle.

f) Nicotine is administered into the blood supply to the lower brain by injecting it into the vertebral artery according to the technique introduced by Magni et al. (1959). Cats are tracheotomized under ether anesthesia and are subjected to section of the spinal cord at C2 and to bilateral cervical vagotomy. The palate vault is trephined, the basilar artery exposed and ligated at a caudal pontine level slightly anterior to the foramen caecum. The right vertebral artery is exposed and cannulated. According to observations by Magni et al. (1959) material injected into the right vertebral artery is conveyed to the medulla, and to the caudal portion of the pons, up to the trapezoid body. The distribution is prevalently, but by no means exclusively, ipsilateral.

For intraventricular administration nicotine HCl is dissolved in Tyrode solution, adjusted to a pH of 7-7.2, kept at from 37.5 to 39 centigrades and is injected in 0.025 ml volumes through a 28 gauge needle which is inserted into the lumen of the 22 gauge cannula. Nicotine injections are repeated at intervals of approximately 1 hour. Between each subsequent nicotine injection 0.025 ml Tyrode is injected as control. Injections are performed within a 30 sec period. The volumes of the injected solutions are well within the order of magnitude of the volumes of cerebrospinal fluid secreted into the cerebral ventricles (0.02 ml/min according to Davson et al., 1962).

Significance:

Injection of very small doses of nicotine into the 4th ventricle elicits marked changes in the level of vigilance of unanesthetized paralyzed cats. To what extent these nicotine effects play a role upon the production of psychic changes occurring in response to systemic administration of nicotine or in response to nicotine uptake during tobacco smoking remains to be seen.

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To be reported at the meeting of the New York Academy of Sciences, New York. April 6-7, 1966.

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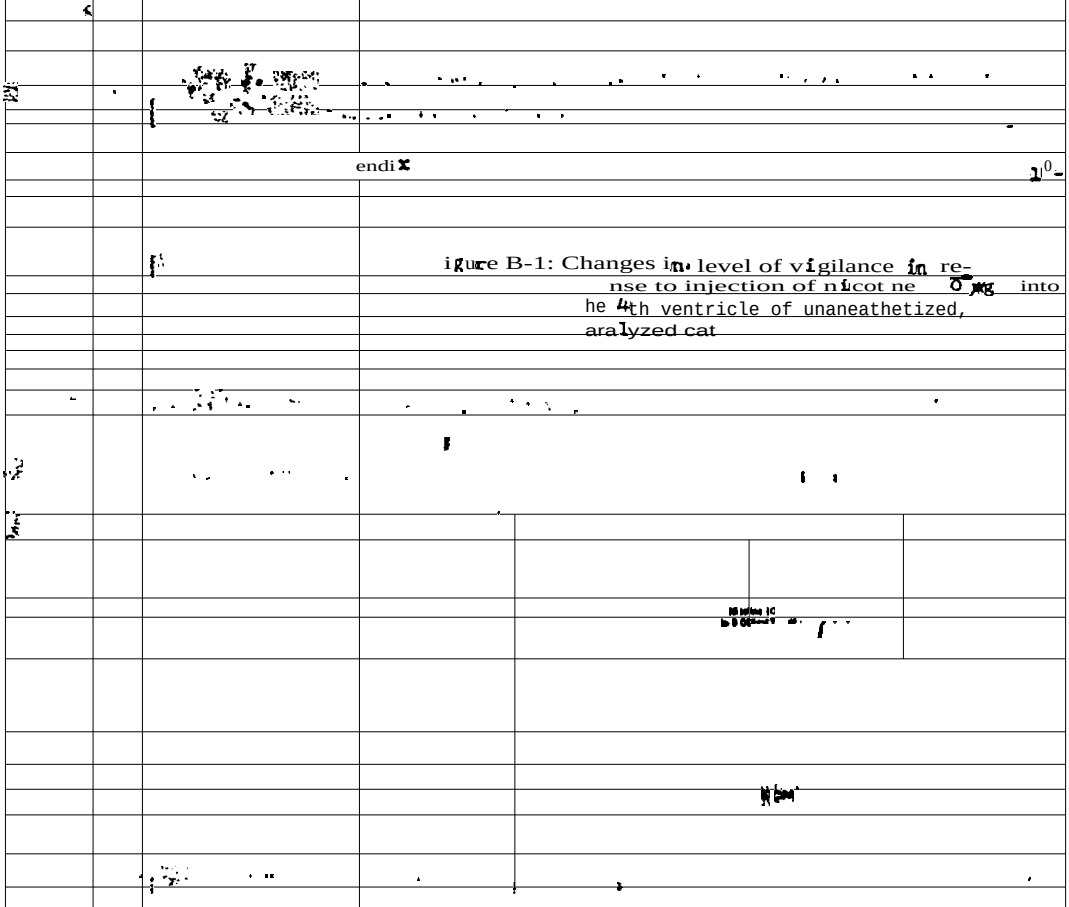
endix



igure B-1: Changes in level of vigilance in re-
nse to injection of nicotine 0 µg into
he 4th ventricle of unaneathetized,
aralyzed cat

Records A, B, C, Top line: tension of nictitating membrane. Second Une: EEG., monopolar recording rom middle suprasylvian gyrus against screw electrode on frontal bone. Third line: frequency analsis of EEG (10 see delay). A: control, B: marker ndicates 30 sec period of nicotine injection, C: eturn to Control level,.

ote: Nicotine injection into the 4th ventricLe f the cat produces contraction of nictitating membrane and EEG desynchronization. These symptoms of rousal lasted approximately 4 minutes.

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Ulrich H. Schaeppi, M. D.

1953 - 1963

1. Dissertation. On the cancerogenic effect of methyl violet (Methylrosaniline Chloride). U. Schaeppi. Dis. University of Berne, 1953.

2. Cholinolytische Wirkung einiger Phenothiazin-, Xanthen- und Anilin-Koerper und ihre Beziehung zur chemischen Struktur. (Cholinolytic effect of some Phenothiazine-, Xanthen- and Anilin- derivatives in relation to their chemical configuration). U. Schaeppi and P. Waser. Arzneim.-Forsch. 8: 107-113, 1958.

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14. ffects of Chlorpromazine upon induced periods of tonic immobility in
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7. Electrical stimulation of the isolated cat iris. U. Schaepp , R. Rubin
nd W. P. Koella. Am. J. Physiol. (In Press).

18. ffect on 1eve1 of vigilance of e nicotine injected into the cat's 4th
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ated cat iris. U. Schaeppi, B. L. Dennison and M. Dodd. J. P rmacol.
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herap. (Submitted for publication, 1/66.)

2. ew observations concerning the iris innervation. . P. Koella and
. Schaeppi. (To be submitted to Science, 1/66.)

3 ffects of drug injection into the cat's fourth ventricle. U. Schaeppi
nd W. P. Koella. (In Preparation.)

new electronic pupil,lometer. B. L. Dennison, U. Schaeppi and
. P. Koella. EEG clin. Neurophysiol. (To be submitted.)

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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#466R1 - HOFF

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CONFIDENTIAL

THE COUNCIL FOR TOBACCO RESEARCH - U. S. A.

February 16, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Ebbe Curtis Hoff, Ph.D., M.D. and Karl C. Corley, Jr., Ph.D. - No. 466R1.

We enclose herewith a grant renewal application from Drs. Ebbe Curtis Hoff and Karl C. Corley of the Medical College of Virginia.

This application covers year No. 2 of a three-year plan that was started with the grant activated on June 1, 1965 with promise of priority consideration for the second and third years. A budget projection was made at $15,000.00.

The committee has received a site visit report by Dr. J. M. Brady dated December 22, 1965 which reflected staffing problems and delays in progress in the project. The present application reports addition of Karl C. Corley to the staff concerned with this study and contains a built-in-progress report.

We are distributing this application at present only to the committee members but plan to include it on the agenda for March 5-6.

R. C. H.

Personal Opinion: The present research plan does seem to me quite relevant to our high-priority interests. However, actual achievements of this group have been consistently thin and staff problems consistently present. Continuity has been notably weak. Perhaps Karl C. Corley will change this. I favor making the renewal but think a close watch should be maintained and the third year made contingent upon substantial progress in 1966-67.

R. C. H.

1005101246

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#466R1
Activated: 6/1/65
Cf. #283
1/1/61 to 1/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Application for Renewal of Research Grant
~~Application for Research Grant~~

Date: February 10, 1966

1. Name of Investigator: (1) Ebbe Curtis Hoff, Ph.D., M.D. (Principal Investigator)
(2) Karl C. Corley, Jr., Ph.D., (Co-Principal Investigator)

2. Title: (1) Professor and Chairman, Division of Psychiatric Research
(2) Assistant Professor, Division of Psychiatric Research

3. Institution & Address: Medical College of Virginia
1200 East Broad Street
Richmond, Virginia

4. Project or Subject: The Effects of Nicotine and its Intermediary Metabolites Upon the Central Nervous System, Autonomic Mechanisms, and Behavior.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

The following represents a Progress Report and additional Plan of Procedure.

The physiological section of the original application was divided into two parts: (1) Acute and (2) Chronic.

With the completion of the acute phases of the experiment during the previous report period, the major effort is being devoted to the chronic studies.

BEHAVIORAL STUDIES

(A) In accordance with the plan of procedure presented in the original proposal, studies on the effects of nicotine and metabolites upon food and water intake are in progress.

On food intake, six male Sprague-Dawley rats are being maintained on a 60-second fixed interval reinforcement schedule. When the baseline performance of the rat stabilize over a three day period with variability of less than ± 10%, the effect of subcutaneous doses of nicotine is tested. At doses as low as 20 micrograms per kilogram, a complete disruption of all behavior is observed 1 - 9 minutes after injection. When lever pressing returns, the level of responding appears to be similar to the control level. Difficulties, however, are encountered maintaining a reliable baseline behavior. At the earliest feasible time, the schedule will be changed to 60-second variable interval reinforcement. This schedule should provide a more reliable behavior, and show a clearer indication of the effects of nicotine.

1005101247

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

etailed Plan of Procedure (Continued from Pg. 1)

(n water intake, baseline data for daily consumption are now being obtaine in six other rat's. The protocol described in the origina.l application is being followed, and the questions raised by the initial work will be explored.

(B) Additional experiments are also planned to study the effects of nicotine and its intermediary metabolites upon discrimination and learning behavior.

Recently an operant avoidance technique has been developed for the study of pattern discrimination in the rat. In discrete trials, random presentation of one of two pattern configurations requires discrimination between a right left lever press to avoid shock.

The apparatus to establish this behavior consists of three Grason-Stadler multiple stimulus projectors for pattern presentation, two Lehigh Valley retractable levers for indication of response, and a grid floor for shock reinforcement. The projectors and levers are arranged so that there is a right and a left panel with a projector and a lever on both sides of a center panel with only a projector

The pattern discrimination obtained in this experiment is maintained by a two phase schedule. The first phase and initial training involves a simple right versus left discrimination. Identical patterns are presented on the projectors of the center and one side panel. The correct response is always a lever press on the side panel showing a pattern. The second phase of the schedule is also presented on the previously unlit panel. This pattern is always different from the other two patterns, and the correct response is a lever press on the side panel showing a pattern corresponding with the center pattern and different from the pattern of the other side panel. In both phases, the presentations are randomized between the two pattern configurations by a pulse distribution.

In rats, highly trained on simple discrimination, the two phases of the schedule are combined to form an alternating mixed schedule. With the same patterns used in each phase of the schedule, every session begins with 15 minutes of the simple differentiation involving two patterns followed by 30 trials of the complex discrimination involving three patterns. Five cycles of this sequence or 150 trials involving three pattern discrimination comprise a session.

Regardless of the phase of the schedule involved, the trials consisted of the following sequence of events. Both levers are introduced into the box, and patterns are presented. In every trial, only a correct response terminates the trial, extinguishing the patterns and retracting the levers. If no correct lever press occurs within 50-seconds, shock is administered at 4-second intervals until the correct response occurs. During complex discrimination, involving three stimuli, the different third pattern is always extinguished after the first shock, thus reducing the configuration to simple discrimination involving two patterns.

During a trial incorrect responsesalways result in retraction of lever press. During complex discrimination and training of simple discrimination, no other consequences result from these responses, but in the simple dis-

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

crimination phase of the mixed alternating schedule, these incorrect responses cause immediate commencement of the shock sequence that continues until the correct lever is pressed.

In fully trained rats, nicotine will be studied to determine its effect upon pattern discrimination. Because this technique uses avoidance procedures, the confounding effects related to the possible anorexic properties of nicotine will be avoided.

In another behavioral situation, the active learning and memory process will be studied. Rats will be trained on a two-lever discrimination. This task requires the association of one lever with food reinforcement and the other lever with no reinforcement. Within each session, the reinforcement contingencies of each lever are reversed after every fifth reinforcement. These reversals require the rat to constantly readapt its behavior pattern by learning and remembering the consequences of the previous lever press.

This adaptability is further emphasized by a delayed response contingency. After each lever press, the reinforcement does not occur until a sensing circuit is activated in the opposite end of the apparatus. At this point, the apparatus lights are extinguished for a specified time out period. If the correct lever was pressed, food reinforcement accompanies the commencement of the time out period, but an incorrect lever press results only in a time out. In both instances, lever presses during the time out are registered as incorrect response and restart the time out. This requires the rat to remember the consequences of its previous response and delay its next response until the apparatus lights are relit at the end of the time out.

In this situation, the duration of the time out has been found to be a critical variable. By analysis of the ratio between correct and incorrect lever presses, this factor can be studied. When the time out is very short (less than 5-second), random lever pressing occurs with no indication that the rat attempts to remember the correct lever from reinforcement to reinforcement. The same behavior is also observed with periods longer than 30 seconds. In between these extremes an optimal behavior is maintained with a time out of approximately 15-seconds.

Because this task provides a measure of the rat's ability to delay or withhold a correct response, a form of memory is being studied. If a compound influences memory, the length of the time that the rat can retain a correct response should be enhanced. Also if learning is affected, the number of correct responses should be reduced.

Previous experiments have shown that the effects of anorexic and stimulant agents can be separated from these other factors. With this ability of differentiation, it should be possible to determine if nicotine possesses any properties to enhance learning and/or memory.

In all of these behavioral situations, any consistent phenomenon found will be further studied with physiological and neurophysiological techniques. This approach makes it possible to explore and determine the neural correlates of these behaviors, and provide data on the mode and site of the action of nicotine.

EEG STUDIES

In cats with chronically implanted electrodes, the studies described in the original application are now in progress. The conditions and procedures previously outlined are being adhered to.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

udget Plan:

a. Salaries	8,800.00
b. Expendable Supplies	2,504.00
c. Other Expenses	None
d. Permanent Equipment	2,000.00
e. Overhead (15% of a,b,c)	1,696.00
Total	15,000-00

7. Antici ated Duration of Work: It is anticipated that the duration of the studies outlinedd under an for Procedure") will continue for at least two more years following the end of present project year, i.e., May 31, 1966.

.. Facilities and SStaff Available: The facilities available for research in our Division include: 1 a fully-equipped laboratory for acute studies; (2) a chronic laboratory with aseptic operating room and histology laboratory; (3) a laboratory with shielded cabinets where electrophysiological studies are conducted; (4) an electronic shop and instrument room; (5) a machine shop; (6) a darkroom; (7) a suite of rooms in which behavioral and neurophysiological studies are combined. The available staff includes the principal investigators, and in addition, three faculty members with special training in neurosurgery, neurology, neurophysiology, neuroanatomy and cardiology. There are also two full-time electronics specialists and three full-time research assistants trained in animal operative procedures.

Standard neurophysiological and behavioral equipment is fully operational, and included Grass and Sanborn physiological recorders, an 8-channel and a 16-channel electroencephalograph, stimulators, stereotaxic instruments, telemetering equipment, apparatus for the construction of microelectrodes along with suitable recording equipment, solid state behavioral programming equipment, etc.

10. Additional Information (Including relation of work to other projects and other sources of support : This research has a significant contribution to make in the elucidation of the neural bases of behavior. A better understanding of the CNS and behavioral correlates of these mechanisms is essential for the interpretation and treatment of many psychosomatic disturbances. The studies outlined in this proposal may help elucidate the role of nicotine in motivation and learning.

All these experiments form an integral part of the continuing research program in our laboratories concerned with cerebral autonomic mechanisms and the regulation of food intake. Additional support is provided by research grants from NIH and ONR.

Signature

irector of Project

Business Officer of the Institution
L. Daniel Crooks, Comptroller

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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CONFIDENTIAL

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

February 15, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application from J. P. Long, Ph.D. - No. 491R1.

We enclose herewith a renewal application from Dr. J. P. Long of the University of Iowa, Iowa City, Iowa. It is accompanied by a progress report consisting of several manuscripts, prepared for publication. The reprint (No. 1 in the list) was mailed to the Board on July 8, 1965. (This paper concerns the iris, not the nictitating membrane).

We have supported this project since 1961. The present application represents the first renewal of a new three year plan for which special consideration was promised.

This application is being sent at present only to members of the committee, but it will be included in the agenda for the March meeting.

R. C. H.

1005101252

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

ARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#4191R1
Activated: 6/1 5
Cf. #302
Activated: 6/1/61
Renewed annually
through 6/1/64

COUNCIL FOR TOBACCO RESEARCH - U.S.A.
SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
630 — D AVENUE
NEW YORK, N. Y. 10017

COMMITTEE
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Renewal
Application For Research Grant

Date: February 8, 1966

1. Name of Investigator:
P. Long, Ph.D.

2 Title: Professor

3. Institution & Address: Department of Pharmacology, College of Medicine
University of Iowa
Iowa City, Iowa

4. Project or Subject:
"Sympathomimetic actions of nicotine." To continue to evaluate the mechanism(s) by which nicotine liberates norepinephrine from various sites. Also evaluate other pharmacological properties, such as the liberation of serotonin (5-HT) from the small intestine.

Detailed Plan of Procedure (Use additional pages if more space is reqwred)

See separate sheet

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

5. The first project will be to evaluate the mechanism by which nicotine stimulates an isolated segment of dog intestine and to evaluate the mechanism of serotonin release. The serotonin release from the sigment is constantly monitored. A diagram of the preparation is shown on the last two pages of section 5. This paper is in press in the Am. J. Physiol.

In our initial experiments 10 μg of nicotine administered into the artery liberates a considerable amount of serotonin. As to whether this produces the intestinal spasm or is the results from spasm remains to be evaluated. At least I do not believe that anyone has demonstrated previously that nicotine can release serotonin from intestinal muscle at this or any other site. Perhaps the released serotonin may play a role in producing intestinal spasm. However, this possible mechanismremains to be established.

Another part of this general problem, is that with this experimental technique hexamethonium (100 μg - 1000 μg), has little or no antagonistic action on nicotine. This dosage of hexamethonium (C-6) is sufficient to abolish the intestinal spasms induced by parasympathetic nerve stimulation. Also this dose of hexamethonium in about one-half of the preparations will potentiate the intestinal spasms induced by nicotine. This lack of effectiveness of C-6 will be evaluated, but this too may be related to the serotonin release.

The mechanisms of the above actions and interactions of nicotine will be studied using various blocking agents for both the autonomic nervous system and serontonin.

Another project will be to continue our studies on the nature of innervation of the cephalic vein in the forelimb of the dog. Venoconstriction is induced in vivo by sympathetic nerve stimulation, acetylcholine, norepinephrine and epinephrine. Nicotine is inactive. The isolated vein is contracted by nicotine, the above agents and by histamine using in vitro preparations. Further studies will be undertaken to evaluate the mechanism in the in vitro preparation.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Since we can not isolate the sympathetic nerves with the vein, we will attempt transmural stimulation to activate the sympathetic nerve terminals. If this is successful then we can evaluate the mechanism by which the various agents produce venoconstriction. Certainly it is unusual that all of these various agents can produce venoconstriction in this very localized area. We have just completed a histololical study and the smooth muscle abundance is remindful of an arterial wall.

I realize that the above outlines are rather brief as to experimental detail, but the experimental approach will be similar to those that we have used evaluating the action of nicotine at other sites. If additional information is needed, I will be more than happy to supply it.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Budget Plan:

Salaries	4,600
Expendable Supplies	900
Other Expenses	45
Permanent Equipment	800
Overhead (15% of a, b, c)	892
Total	7,642.

Travel $300.00 + $150.00 for publication costs

Anticipated Duration of Work:

July 1, 1966 - June 30, 1967

Facilities and Staff Available:

Presently I am working in an approximately 450 ft2 laboratory. Some time this fall I will obtain an additional laboratory of about 300 ft2. The laboratories are air conditioned and recently remodeled i

Additional Requirements:

None anticipated

0. Additional Information (Including relation of work to other projects and other sources of support):

In addition to the council for Tobacco Research - U.S.A., my research is funded by the following sources.

Source	Title of Grant:
MA-ERC	Cardiovascular and Centrall Nervous System action of Nicotine Congeners.
IH	Studies on Cholinergic Transmission
IH	Structure Activity Relationships of Autonomic Agents.

The NIH expenditures are kept entirely separate from the nicotine grants, but there is some joint support of research from the two sources of money for support of research in nicotine.

Signature

Director of Project

Business Officer of the Institution

TREASURER

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#492R1-BOOKER

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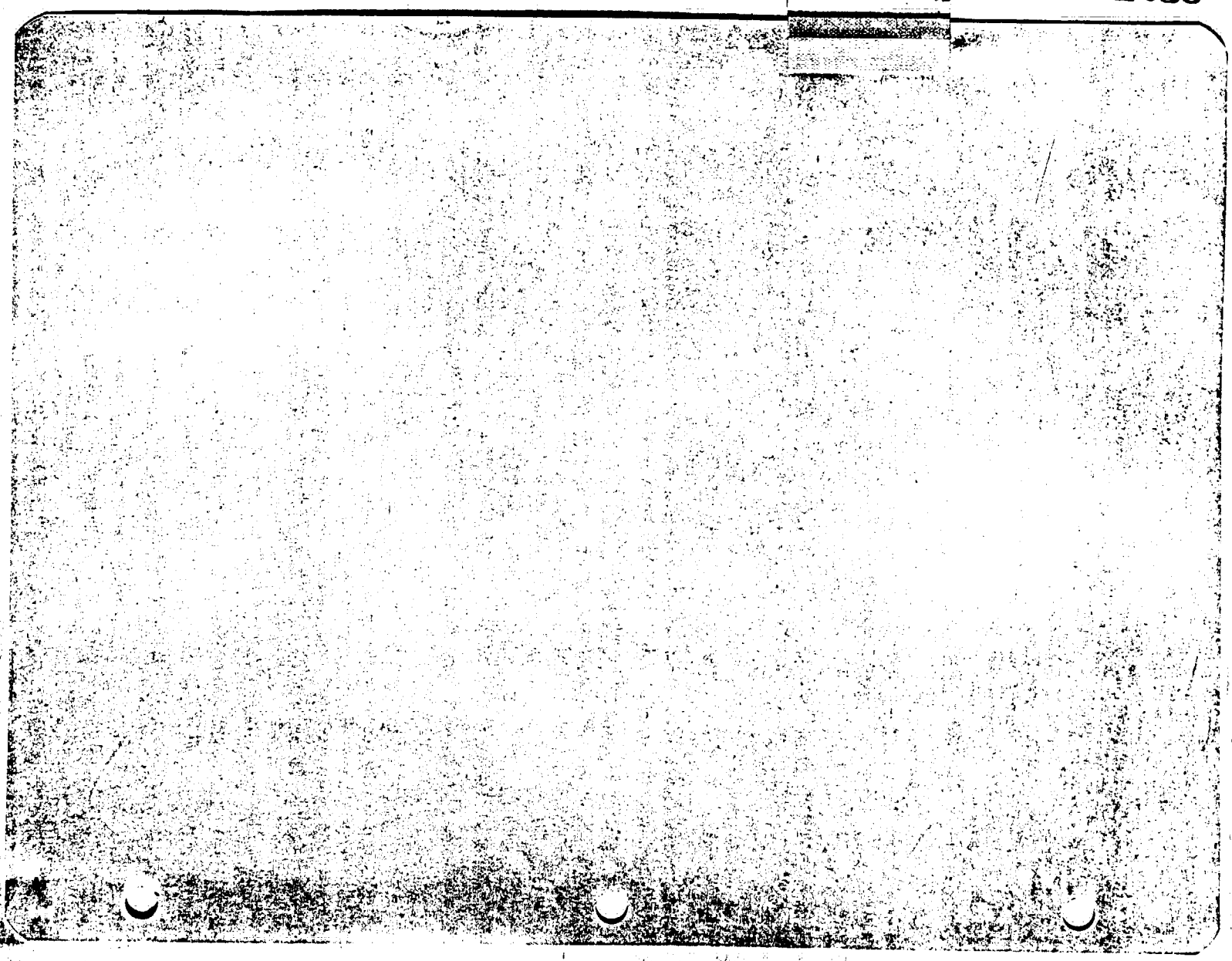

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH, U.S.A. CONFIDENTIAL February 18, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Walter M. Booker, Ph.D. - No. 492R1.

We enclose herewith a renewal application from Dr. Walter M. Booker and his colleagues of Howard University in Washington, D.C. A progress report is attached.

Dr. Booker applied in April 1965 for three year support. The grant was activated on July 1, 1965 for one year without further commitment, but special consideration was promised for the first and second renewals, following review of progress.

This application is being sent at present only to the committee but it will be included on the agenda for March 5-6.

R. C. H.

1005101260

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

HE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

UCCESSOR TO THE
OBACCO INDUSTRY RESEARCH COMMITTEE

OMMITTEE:
r. Cattell, Chm.
r. Bing
r. Jacobson

633 THIRD AVENUE
NEW YORK, N. Y. 10017

Renewal

pplication For Research Grant

ebruary 11, 1966

. Name of Investigator: Dr. Walter M. Booker, Dr. S. N. Pradhan, and r. T. A. Balourdas

Title Professor & Head, Department of Pharmacology, Professor and ssistant Professor, respectively.

. Institution & ddress: Howard University
520 W Street, N. W.
Washington, D. C.

. Project or Subject:

"Studies on the Possible Sensitization of the ascular Mechanism to Catecholamines Following Nicotine Administration"

. Detailed Plan of Procedure (Use additional pages if more space is required.)

urrent Experiments and Experiments Planned for the Next Year:

t present the experiments reported in the abstracts (attached) are eing conducted along the lines mentioned. We plan to expand these experiments as various developments occur. In addition to these experiments, we lan to start the following experiments:

1. Cardiac catecholamine depletion by daily injections (every three ours or more often) of nicotine in normal and scorbutic guinea pigs.

2. Influence of nicotine on resynthesis of cardiac catecholamines in ormal and scorbutic guinea pigs.

3. Sensitivity of the isolated hearts of uinea pigs to norepinephrine nd to angiotensin following nicotine administration (perfused for 10 to 5 minutes).

4. The responses of hypertensive dogs to nicotine doses; comparison ith normotensive responses.

10 01 9

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

In vivo experiments have shown the development of vascular hyperreactivity induced by nicotine. The observations were made on the microvascular system of rats by direct visualization of the minute mesoappendiceal vasculature under the microscope after administration of nicotine (35-40 µg/kg). (See copy of abstract attached.)

Further experiments are designed to determine whether or not nicotine administered to animals subjected previously to abnormal pathophysiological conditions causes the same effects on the microvessels, as above mentioned, or its effects are more prominent, different and/or exaggerated. Specifically, the rats should be subjected to experimental diabetes mellitus by alloxanization or by pancreatectomy with the possible development of microangiopathy, as is expected in diabetic conditions.

Using the same bioassay technique of the rat mesocaecum preparation, we shall be able to obtain direct observations of the effect of nicotine on two groups of experimental animals, viz.

(a) Rats treated with nicotine previously being normal, and used as control groups; and

(b) Rats diabetic become experimentally used to test the effect of nicotine administration on already hypersensitive capillary vessels

The development of vascular hyperreactivity and other morphological and functional changes in abnormal conditions may have several clinical implications and many morbid entities should be explainable by their existence. Secondary adjunctive potentialities might be precipitated and aggravated by predisposed hyperreactive blood vessels and vice versa. Such hypersensitive vessels appear more hyperreactive to normally and abnormally discharged and circulating endogenous amines as well as to exogenous vasoactive agents and factors.

Further studies on the responses of the blood pressure and the somatic reflexes to various doses of nicotine injected into lateral ventricle are intended in future experiments. These studies are to be extended after administration of the compound into the third and the fourth ventricles and also in the various parts of the lateral ventricle as have been demonstrated in the case of d-tubocurarine by Feldberg and his associates. Microelectrophoresis of nicotine into several subcortical sites may also be attempted.

On blood pressure, the pattern of response needs further confirmation and analysis of the response into possible components is intended by usual physiological and pharmacological means.

Studies on the effect of nicotine on reflexes will be extended to other somatic and autonomic reflexes involving supraspinal sites. Its effects on some central structures, such as reticular formation, hippocampus, hypothalamus and others will be investigated by studying the changes in the responses of blood pressure, various reflexes or other physiological functions induced by stimulation of these structures after central administration of nicotine.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

This work will be organized in the same way as currently organized; that is, sections of it will be pursued under the direction of the staff persons named in this grant. Graduate students, research assistant (now employed) will be used in the work.

Papers Published or Presented at National Meetings:

"Blood Pressure Responses to Epinephrine and to Norepinephrine Following Nicotine Administration"

Presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics. This paper is now being prepared for publication.

Experiments pursued during the past six months:

The attached abstracts of papers which will be presented at the Federation meetings in Atlantic City, April 9-15, 1966 can best describe the work that has been in progress.

BUDGET

BUDGET	
Personnel	
Research Assistant GS-6	$ 5702.00
Staff Benefits	342.00
Expendable Supplies	
Animals, Glassware, etc.	3000.00
Other Expenses	
Student Stipends & Tuition	4347.00
Travel	500.00
Other contractural services	200.00
Permanent Equipment	1000.00
Overhead	2114.00
Total	$17205.00

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$ 5702.00
b.	Expendable Supplies	3000.00
c.	Other Expenses	5389.00*
d.	Permanent Equipment	1000.00
e.	Overhead (15% of a, b, c)	2114.00
	Total	$17205.00

*See itemization of "other expenses".

7. Anticipated Duration of Work: Two years.

8. Facilities and Staff Available: Well-equipped laboratories, animal quarters, apparatus for cardiovascular and neuropharmacological and other types of measurements. Spectroflurometer for estimating catecholamines. Facilities for studying numerous isolated organ preparations.

Staff is active in research. Graduate students take part in research with and under direction of the staff.

9. Additional Requirements:

10. Additional Information (including relation of work to other projects and other sources of support):

Same as indicated on original application.

Signature [signature] Director of Project

[signature] Treasurer
Business Officer of the Institution

100510264

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

CONFIDENTIAL

February 15, 1966

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., and Dr. Little. (Dr. Seltzer)

FROM: Robert C. Hockett

SUBJECT: New grant application from Norman W. Heimstra, Ph.D. - No. 527.

We enclose herewith a new grant application from Dr. Norman W. Heimstra of the University of South Dakota at Vermillion, South Dakota.

This application proposes a new study to follow up on the findings of his first eighteen month study (#421) on the Effects of Smoking on Sustained Performance in a Simulated Driving Task, which was activated on June 1, 1964.

A draft copy of his final report on the first study is enclosed.

This application is being sent at present only to members of the committee but we plan to include it on the agenda for the March 5-6 meeting.

R. C. H.

1005101266

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC

NO. 527

Cf. #421
Activated: 6/1/64

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Little
(Dr. Seltzer)

Application For Research Grant

Date: January 25, 1966

1. Name of Investigator: Norman W. Heimstra, Ph.D.

2. Title: Assoc. Prof. Psychology, Director, Driver Behavior Laboratory

3. Institution & Address: University of South Dakota
Vermillion, South Dakota

4. Project or Subject: An investigation of the relationship between smoking deprivation and stress

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

(See attached proposal)

1005101267

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$6800
b.	Expendable Supplies	900
c.	Other Expenses	550
d.	Permanent Equipment	
e.	Overhead (15% of a, b, c)	1237
	Total	$9487

7. Anticipated Duration of Work:

June 1, 1966 - December 31, 1967 (18 months)

8. Facilities and Staff Available:

Facilities and staff are available to conduct this investigation. Direct supervision will be by Dr. Heimstra. Staff at the medical school will be available for consulting and for analysis of blood and urine. Tracking and reaction time equipment will be constructed. All other equipment is available.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of support):

The proposed investigation relates to other work being conducted in the laboratory which is being funded by government agencies. Closely related are projects on the effects of environmental stress on performance which are currently being conducted. Procedures and equipment utilized on these investigations can be utilized on the proposed study.

Signature Norman Heimstra
Director of Project

Fred E. Marshall
Business Officer of the Institution

100510268

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

n Investigation of the Relationship Between Smoking Deprivation and Stress

NTRODUCTION

In a recently completed investigation at the Driver Behavior Laboratory at the University of South Dakota, the effects of smoking on performance in a simulated driving device was investigated.[1] The experimental design involved three groups of subjects: a non-smoker group, a smoker group and a smoker-deprived group, While few differences in performance were noted in the case of the smoker and non-smoker groups, performance of the deprived smokers was consistently inferior to that of subjects in the ther groups. There were several indications that subjects in the smoker-deprived group were under considerably more stress than subjects in either the smoker or non-smoker groups.

While no direct evidence was obtained to verify the possibility of a higher level of stress in the deprived group, their behavior strongly suggested this. In several instances subjects refused to continue with the driving task even though they were aware that they would not be paid if they did not continue. Also, several subjects became quite aggressive in an overt fashion. Comparison of the data obtained from the smoker-deprived group showed that is was very similar to data obtained in an investigation in this laboratory where subjects were intentionally stressed by means of electric shock. In addition, comments made by subjects after completion of the task indicated that they considered it to be stressful.

Although considerable research relating smoking with various behavioral and physiological measures has been accomplished, little interest has been shown in the possible effects when individualsare intentionally deprived ofsmoking. This is.

specifying withdrawal effects of many other types of chemical gents.

The purpose of the proposed investigation is to determine, by means of behavioral and physiological measures, the effects of smoking deprivation on "light"' and "heavy" smokers.

METHODS

Subjects

ubjects will be male college students recruited from

[1] Supported by a grant from the Council for Tobacco Research, U.S.A.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

various classes at the University. Subjects will be paid to participate in the investigation.

Students will be requested, in their classroom, to fill out a short questionnaire about their smoking habits. While a number of questions will be included, the items of primary importance for this study will be whether the individual smokes, ever smoked, the frequency of smoking and whether he inhales regularly. On the basis of this questionnaire, potential subjects will be classed as non-smokers, light smokers and heavy smokers.

The difficulties of a classification of this type is recognized and has been discussed by Larson, Haag and Silvette.[1] However, in this study a light smoker will be defined as one that smokes between five to ten cigarettes a day and indicates that he inhales regularly. A heavy smoker is operationally defined as one that smokes over 20 cigarettes daily and inhales regularly.

Subjects, on the basis of their replies to the items on the questionnaire will be contacted as possible subjects--they will be approached by phone and again, in a casual fashion, asked several questions about their smoking. If their answers regarding the frequency of smoking and inhalation correspond to their questionnaire responses they will be asked to serve as subjects.

Health records of all subjects selected will be routinely examined to screen for any obvious physical defects--all subjects will be paid at the rate of $2.00 per hour for a six hour session. A minimum of 15 subjects in each of five groups will be utilized for a total of at least 75 subjects.

Procedures

Subjects will be assigned to one of five groups: a non-smoker group, light and heavy smoker groups, or light and heavy smoker-deprived groups. There will be 15 to 20 subjects in each group.

All subjects will perform on a task which involves tracking and reaction time. They will be tested for six consecutive hours. The tracking task requires a coordinated motor response to a continuously varying display. It is probable that the tracking task utilized in the proposed study will involve the subject keeping a marker on a target, whose position is changed in a random fashion. This is accomplished by means of an aircraft-type control stick. Depressing a button on the control will be the response in the reaction time test. The response will be made to a multiple list of stimuli. Subjects in the deprived groups will have all cigarettes removed prior to testing. Subjects in the smoking groups will be furnished with cigarettes. For these groups, cigarettes will be placed in a container in front of

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1 "Measurement of Tobacco Smoking", Medical Times, Vol. 88, No. 4, April, 1960.

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hem. An automobile type lighter will be furnished. This system entails the least amount of physical manipulation which might interfere with the performance tasks. In addition, by means of closed circuit television system, the subjects will be observed ata will be obtained concerning the performance on the tasks prior to, during, and after smoking. lysis of these data will ield someinformation concerningi the effects of smoking ona psychomotor task.

rior to being placed in he test apparatus, blood will be aken from each subject by pricking his finger. This willlalso one after the six hour test session. Similarly, a urine ample will be taken before and after the session. Blood pressure will also be taken before and after the session.

n the subject is seated in the device various leads from olygraph will be attached. A shoe and' sock will be removed from the left foot and the foot will be strapped loosely into a holder which will prevent gross movements. The pickup for a lethysmograph will be attached to the big toe. Pickup electrode for a GSR will be attached to the sole of the foot or ankle. An attempt will be made to'obtain skin temperature from a pickup attached to the lower calf of the leg. Respiration will be easured from an abdominal pneumograph. Muscle tension changes will also be recorded.

time sampling procedure will be utilized with the above easures. Recordings will be taken during two five-minute sessions each hour. The following measures, then, are obtained rom subjects in each group during the test session.

erceptual-Motor Tests

Tracking--This is a fairly sensitive measure. Performance on tracking tests has been shown to be impaired'by a number f stressors such as cold, heat, fatigu , acceleration and confinement. Scores involving duration "off target" and frequency ff target are obtained

Reaction Time--This measure has been used extensively tress experiments. Since simple reaction time has shown ittle sensitivity to stress, a complex reaction time task, involving several signalss will be used. It has been found that where several signals are used and the subject must choose the roper stimulus before reacting, stress sensitivity is increased

hysiological Measures

Urine Analysis--Urine samples taken before and after he test session will be analyzed for catechol amines and adrenal steroids. These appear to be relatively sensitive indices of stress.

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2. Blood Pressure--While not a particularly sensitive indicator of stress, a before and after measure might be revealing. Because of its simplicity it is included.

3. Blood Count--There is considerable evidence that a reduction of eosinophils takes place after exposure to stress. Pre- and post-test samples will be taken and comparisons made.

4. Respiration--Results in stress studies have been varied. There is some evidence that respiratory rate increases in emotional arousal.

5. Plethysmograph Readings--Degree of constriction or dilation of peripheral arterioles is an indicator of sympathetic activity. Evidence that blood volume change is related to stress. Also, pulse rate is obtained from plethysmograph readings.

6. Skin Resistance (GSR)--There is considerable work on this measure and it appears to be a relatively sensitive indicator of stress.

7. Electromyograph--Records action potential of skeletal muscle. With increased muscle tension in stress, changes in action potentials are found.

8. Skin Temperature--Stress is reported to produce a fall in skin temperature.

While the number of measures utilized may appear excessive, past experience has shown that a battery of variables is desirable. Even a battery of measures cannot always effectively measure the arousal value of a stressful situation. However, if a number of measures are utilized and a large number of subjects are used, physiological variables that appear to be more sensitive to a given stressful situation for most of the group can be isolated and evaluated.

Data Analysis

Differences between the groups in terms of performance on the tracking and reaction time measures can be tested by means of analysis of variance to determine significance. Analysis of differences between groups and over time will be obtained. Data obtained from several of the physiological measures are also amenable to this type of analysis. Primary evaluation of physiological data will involve comparison of changes in individual subjects over time. However group comparison will also be made.

SIGNIFICANCE OF STUDY

As pointed out previously, little interest has been shown in the effects on individuals of deprivation in smoking. It would seem that this aspect of the total behavioral pattern

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associated with smoking is of significance and may have more striking effects than smoking itself. It can, perhaps, be viewed in the same context as "withdrawal" in the case of other drugs.

As designed, the proposed study should yield information concerning the effects of smoking deprivation in terms of stress or emotional arousal associated with the situation. This will be reflected in physiological measures as well as performance measures. Also, the design is such that the relationship between the degree of normal smoking (i.e., light and heavy) and stress reaction can be obtained.

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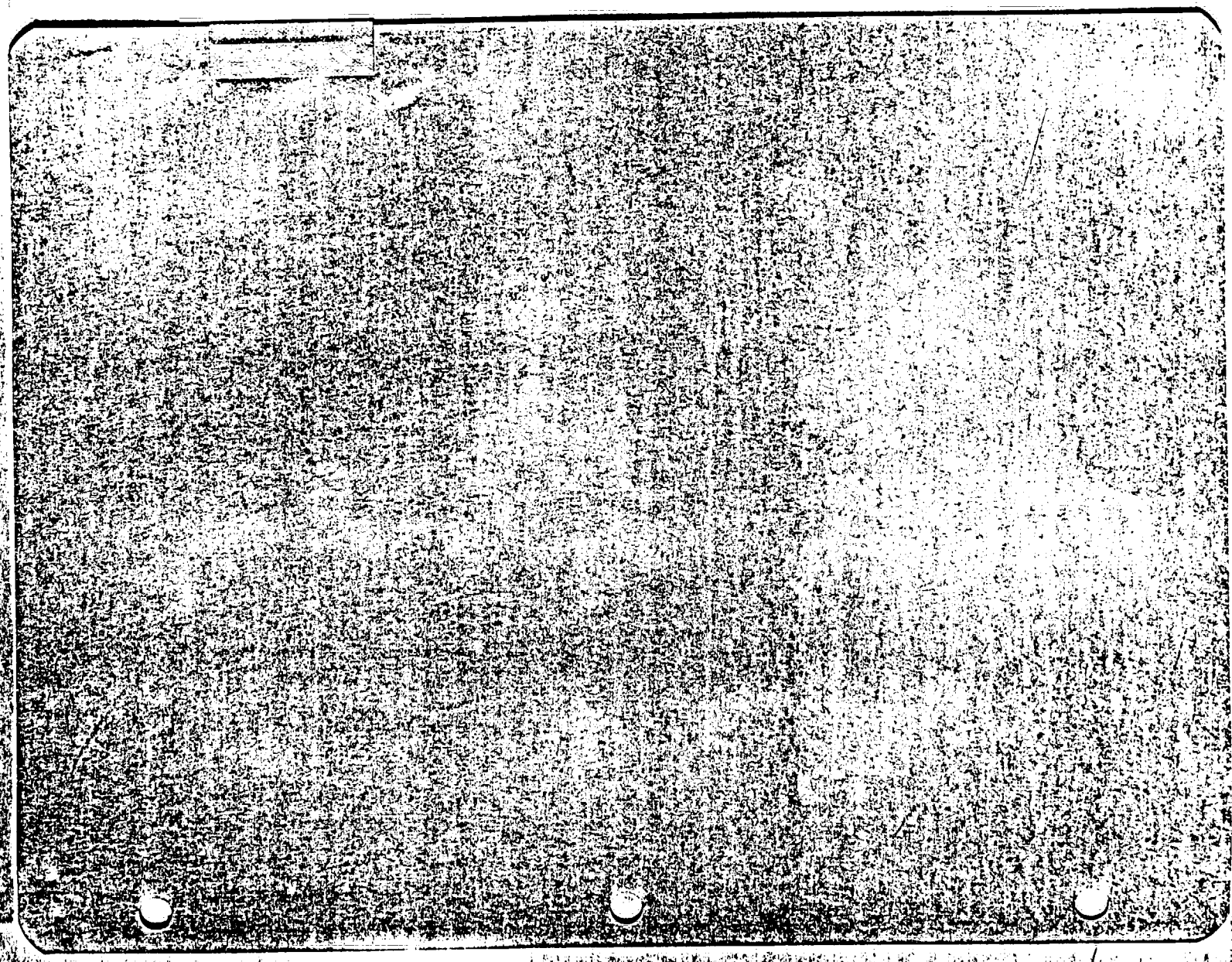

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COUNCIL FOR TOBACCO RESEARCH

ebruary 14, 1966

O: The committee comprising Dr. Jacobson, Chairman, Dr. ing and Dr. Reinmann.

ROM: Robert C. Hockett

UBJECT: Application for a grant from Sue Buckingham, M.D. - #528.

e enclose herewith a grant application from Dr. ue Buckingham of Columbia University. t is classified as a ew grant since the last grant completed the three-year period riginally requested. A report of progress is incorporated in he grant application.

he last grant was made without further commitment. he present application proposes a new three to five-year program.

This application is being sent at present only to embers of the committee, but we plan to include it in the agenda for the March meeting.

C.H.

CH:ek

ncl.

ersonal Opinion: This appears to be a ood research program, nd I would favor it as a systematic approach o the basic problem of surfactants and their elation to pulmonary disease..

10.

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PHYSIOLOGY OF THE RESPIRATORY SYSTEM

No. 528
Cr. #385R3
Activated: 6/1/6
Renewed: 6/1/64
Renewed: 7/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Bing
Dr. Reimann

Application For Research Grant

Date: January 17, 1966

1. Name of Investigator: Sue Buckingham, M. D.

2. Title: Assistant Professor of Pediatrics

3. Institution & Address: Columbia University, College of Physicians and Surgeons Babies Hospital (Box 70), 167th Street at Broadway, New York, N. Y. 10032.
Laboratory: William Black Research Institute, Rooms 417-418, 630 West 168th Street, New York, N. Y. 10032.

4. Project or Subject:

The Development and Biology of Lung Surfactant (Year IV).

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Many laboratories in this country are considering lung surfactant as their major interest; nonetheless, in the last calendar year, there has been little advance in sound working information about synthesis and turnover of this interesting phospholipid. Harlan's investigation in dogs with C-14 labeled palmitate linked up the production of surfactant with lung phospholipid turnover, and demonstrated that the half-life of palmitate label was shortened by the experimental production of pulmonary edema (9). Pursuing a different approach, our group made two observations: 1) the appearance of both acetate and palmitate into phospholipid moieties of lungs is incredibly rapid, especially with acetate precursor! 2) this anabolism is associated with the large alveolar (septal) cells exclusively, as radioautographs showed. (1) Taken together, these two investigations inferentially link dipalmityl lecithin formation with lung surfactant production, and underline the truly incredible metabolic machinery contained in these cells. Macklin, of course, termed the septal cells "a diffuse organ within an organ". (10) Unpublished data obtained by the Yale*(Gluck) and Morgan (Washington) have begun to dissect the metabolic pathways of lung phospholipid synthesis. These represent the first efforts to outline enzymes that may be involved in synthesis of this disaturated phosphatide. There appears to be a definite relationship between phosphatidylethanolamine and phosphotidylcholine pathways in lung. Morgan thinks two specific transferases are involved and is isolating them. Felts (California) continues to investigate lipid handling by lung, including the fashion in which precursors are extracted from the circulation. (11)

* group

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Beyond finding that phospholipid labeled with tritiated acetate occupies a geographic position in the alveolar cell cytoplasm other than the special inclusion bodies -- which indicates to us that PL ynthesis may occur outside of these organelles -- d to further clarify the relationship of the special inclusionfbody toa surfactant phospholipid format ion., It is hoped that ellectron microscopic autoradiography of lipid soluble materiali wilt be more satisfactory in the future. Following our report that the inclusions were alkaline phosphatase positive (indicating their non-mitochondrial nature) (2), Canadian workers described in newborn lungs a cytoplasm lamellar body from which they thought the inclusions developed. The granule" was acid phosphatase positive. This might indicate one of two possibilities: I) early secretory granulies coming off Golgi (which are acid phosphatase posiitive, according to Novikoff). The author's interpretation, however, was that the material was 2) a degenerative autophagic inclusion of some sort, perhaps related to the lysosome. (12 1 We, and also Campiche (13)', have now Identlled these precursor bodies morphologically. Our failure to obtain acid phosphatase activity in the mature inclusions corresponded to their own findings.

It does seem, however, that most of the important biological uestions about the alveolar cell remaln elusive. Among the major unanswered questions are the following: 1) its derivation, whether entodermal or mesodermal;. 2) its relationship, if any, to the alveolar macrophage; 3) the biological role and identiity of the alveolar cell nclusion bodies; 4) the answer to the question Macklln asked regarding the muco id nature of the alveolar lining subs tance; 5) information, regarding any controlling mechanisms of the production or. Inhibition of surfactant phospholipid, ei ther Ins ide or outside the cell; 6) whether pulmonary edema develops from damage to the cel I or Its product, or occurs the other way around, or both; 7) whether its inhibi-, tion In Immature lungs is a reflection of failure of production, functional depl et ion, or inhibit ion by, say, e xogeno us protei'ncontaining materials in the alveolar space.

If experience continues to repeat itself, some projects in our investigation wi II move rapidly toward a successful conclusion, while others are gestating various technical difficulties. For this reason, era i aspects of a general quest ion wi 1 1 be In some stage of prosecion at any given iime.

The work beingi outlinedwil l itself constitute a kindi of progress rt, since each study has progressed some (variable) distance toward publicat ion. Those mentioned first have accumulated the most data.

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1. The pathogenesis of stress-induced pulmonary lesions leading to surfactant inhibition: high oxygen pressure (HOP). (Buckingham, Sommers, McNary)

We assume, but do not know, that the large alveolar cell is generically trophic, i.e., it will respond to nervous stimuli or to neurohumors released in the immediate vicinity. We previously suggested (3) that the pathogenesis of the alveolar hyaline membrane in infancy might involve "hypersecretion" of the respiratory epithelium. When Jamieson and van den Brenk (14) reported surfactant inhibition by high oxygen pressure in rats that was modified by the action of barbiturates, we considered that HOP pulmonary edema might result from direct neurogenic stimulation of the surfactant producing alveolar cell. Knowing that some systemic stresses deplete catecholamines in the brainstem (presumably by increasing the functional activity of sympathetic neurons), it seemed theoretically possible that such a situation might obtain in HOP and, moreover, that prolonged central discharges might lower neurotransmitter (NE) stores in peripheral organs, including the heart and lung, and thus alter the normal output of lung surfactant. This particular study was high in priority because high oxygen pressure had been suggested as a treatment of respiratory distress syndrome. Even at the outset we felt this treatment to be contraindicated, inasmuch as we suspect central autonomic centers are tentative in these infants, and might suffer unduly from an agent known to be a central nervous system toxin.

The data obtained to date, which have been reported at the Columbia Medical Society (December 1965) and will be discussed at Federation meetings (4), have approached the question of the pathogenesis of the lung lesions by way of their central nervous system connections. A theoretical framework for stress was established by Vogt (15) and also by Maynert (16).

Adult rats exposed to 75 psi gauge pressure (5 atm) for 30 minutes were found to lose more than 50% of their brainstem norepinephrine, and somewhat more than that from the myocardium (65%). After only 15 minutes (an exposure that produces no clinical symptoms in animals), 25% of the brain catecholamines are already lost. The stress-induced loss of NE in both the brain and heart was completely prevented by pretreatment with 75 mg/K of pentobarbital.

Furthermore, lung weights of barbiturate-HOP animals remained normal, and lesions to be described did not develop. Barbiturates are known to interfere with the reticular activating system, and its mode of action here may be to inhibit afferent impulses to the hypothalamus. Pentobarbital also interferes with recovery time of autonomic ganglia, suggesting a second mechanism. (8)

It was not feasible to measure norepinephrine depletion in the lung, inasmuch as tissue concentrations are too low to assess changes with reliability. We plan to study lung NE stores by means of fluorescence microscopy (Falck). (17)

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We were interested to sfudy the effects of i-OP' on lung serotonin (5 HT) for several reasons: rodents s tore great. quantities of' this amine. In their lungs, and have been suggested as a "serotonin reactive lung species". (18) Unlike guinea pigs, whose lungs contain a high concentration of histamine and to which amine they react, rats and mice tolerate injected histamine that would be many times over fatal! to guinea pigs or to man. (19) However,, rodents respond to serotonin. i's a mediator, furthermore, and is a very potent pulmonary hypertensive agent in all species studied. Its role as a mediator has been suggested in endotoxin administration (20) and in the pulmonary hypertension associated with experimental microemboli. (21)

In our experiments (·5 atm HOP 30 min), lung serotoniin content was reduced to 50% of its normal levels. As with the catechol a- stores, barbiturate premedication somewhat-prevented these 5 HT losses in lungi, but was not completely protective. We have identified the cellular site of serotonin storage in rat lung by means of autoradiography following intravenous administration of tritiated 5 HT (5)I. In other viscera, mast cells of rat lung selectively accumulate grains. Using other methods, Rowley and Benditt had previously found that serotonin in rat skin was partiially contained in mast cells. (22) Mast cells are very abundant' in rat lungi and are, for the most part, ocated in the adventitia of small vessel

en I-OP-treated lungs were stained' with Giemsa to identify mast cells, It was apparent that lung serotoni'n release must involve losses from endogenous mast cell' stores, inasmuch as these elements were often almost completely degranulated. In contrast, the lung mast cells of barbiturate-protiected HOP animals.contained' abundant numerous healthy granulated mast cells..

Another source of the serotonin released in lung might be from rculating trapped platelets (vidie infra).

Lungs were examined by light and electron microscopy following various dosages of HOP, ranging from 45 to 75 psi pressure, along with barbiturate-treated HOP, and with normal control lungs. Theprimary lesion in the lungs o HOP animals occurred in the smallest ulmonar venules. This was first seen in a 4'5 psi anl mal and, To a modest e- ee, in a 75 psi pentobarbital-treated 1-OP' animal. The lesion consisted of palisading of white cell's and platelets onto.the endothelial surface, hyaline degeneration of the venular wal I, and. the coI Iection of fluid and cells in the perivenous lymphatiics. This clearly indicates that this may be the primary site of high oxygen pressure pulmonary injury, and celils In the lymphatics Indicaife selective leakage at this point. This is of great interest, becavse MiaJ no and Palade (23) have found that several mediators of vascular permeabiility, including sero- tonin, bradykinin, and histamine,. select the post-capillary venule in other organs as the primary site of leakage, and Rowlley has forwarded idence to suggest that' smooth muscle contraction precedes production of the leak. (24')

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Whether released serotonin from mast cells is a mediator in the venular lesion must remain speculative. It is perhaps more likely that an interaction on the venular surface between endothelium and the platelets/WBC may stimulate the release of factors that aggravate inflammation, one or more of which may act to degranulate the mast cell granules. We do not suggest that mast cell 5 HT depletion occurs by way of a neurogenic impulse. Uvnäs believes that histamine release from mast cells by small molecular weight releasers, such as 48/80, unmasks a phospholipase A contained in the mast cell membrane which then itself releases the amines. (25)

It is not known whether 5 HT release from undamaged platelets occurs in vivo but, in vitro, quantitative release of serotonin can be brought about by very low outside concentrations of either trypsin or thrombin (6), but by no other enzymes studied. (7) Furthermore, amine release does not affect the morphology or the metabolic efficiency of the platelets. Generation of tiny amounts of thrombin during the trapping of platelets in the venular and capillary walls of the lung following exposure to HDP can be inferred from examination of other small vessels in which blobs of profibrin were present. It is possible that this mechanism could account for release of small amounts of platelet serotonin into the vessel wall and instigate subsequent lesion and leakage from the venule. Although histamine content was not studied, its activity as a mediator in this situation is less likely -- even though it is present in mast cell granules of the rat -- because of the great tolerance rodents have for histamine.

In the severely damaged edematous HDP lung, a series of vascular changes, ranging from venular damage to platelet and fibrin thrombi in capillaries, venular and periarterial edema, congestion, and even exudation of fibrin into the alveolar walls through damaged alveolar capillaries and venules, were present in various parts of an individual lung. The large alveolar cells were normal-appearing except for one bizarre feature, namely, the development of a large cytoplasmic bleb on the air face that was filled with endoplasmic-like structures. Such a lesion has not been previously described. Microvilli were present on the air surface of alveolar cells, and the numbers of cytoplasmic inclusions were reasonably normal.

The surface activity of high oxygen pressure lung extracts was tested on the Wilhelmy ballance and, after 30 minutes (75 psi), all lung extracts were normal. Even after 1 hr., two/seven extracts retained their normal surface activity. Thus, we must conclude that surfactant inhibition in this situation is probably not a direct effect on the alveolar cell, but may occur secondary to the leakage of fibrinous exudate into the alveolar space that could form even a coacervate with the surface active lipoprotein. (26)

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Bean (27) hypothesized that the sympathetic nervous system was somehow involved with the toxicity of high oxygen pressure since adrenal medullectomy, as well as chlorpromazine pretreatment, were protective. However, these are the first data that speak directly to the Bean hypothesis and they support it. Furthermore, they indicate that peripheral effects of what we presume is a CNS discharge include the myocardial catecholamines (and ?lung), and even epinephrine secretion from the adrenal medulla. The effects of increased circulating catecholamines may help to exacerbate the venular lesion in the lung.

We will also study with Dr. T. Omura whether abnormality in the electrical impulse of the myocardium results from discharge of NE granules from the cardiac nerves in the heart. We plan to examine the amine depletion in this stress system by means of fluorescence microscopy, as well as by chemical analysis. This will permit the localization of stores in affected nerves.

II. The mechanism of pulmonary edema and surfactant alteration induced by vagotomy. (Buckingham, Sommers, Goldenberg, McNary) (8)

Klaus et al (28) in 1961 reported that bilateral vagotomy destroyed normal surface activity of lung extract, and reduced the number of inclusions in the large alveolar cells (4-6 hrs.). Decreased numbers of inclusions that corresponded in time with surfactant alteration also came from exposure to CO_2 intoxication (24-48 hrs.). (29) Surfactant alteration occurs 1 hr. after high oxygen pressure; this makes HOP the most rapid experimental means to produce experimental alteration of surfactant in adult animals.

Vagotomy-induced edema remains of interest for those concerned with the pathogenesis of hyaline membrane disease, because Miller reported that hyaline membranes develop in bilaterally vagotomized animals. (30) We also have found a lesion in the nucleus of the vagus nerve in the brainstems of these infants, as well as in some with fetal pulmonary hemorrhage. (9)

Cervical section of the vagus nerves produces widespread changes, of course, not only in efferent autonomic control but disturbs afferent stimuli, many visceral fibers of which travel in the vagus nerve. Numerous studies of the mechanism of pulmonary edema formation in this circumstance have reached divergent or inconclusive opinions. Some workers doubt that parasympathetic nerves are even present in pulmonary vessels, although Hebb (31) has demonstrated cholinesterase in small pulmonary arterial ganglia that must be parasympathetic (since sympathetic ganglia are all located in the paravertebral region). Kraus feels from in vivo studies, utilizing thoracic windows, that the vagus controls the pattern of intermittent flow observed to involve entire lung sublobules at normal lung volumes. (32) Farber, earlier, concluded that parasympathetic nerves to the pulmonary vessels might be vasoconstrictors. (33)

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At any rate, the final effect of vagotomy would be to Inc

may resemble in some respects the HOP situation previously discussed in which prolonged central sympathetic discharge may set the stage for high oxygen pressure pulmonary ede

chnique of Majno and Palade (23) to outline he sequence of pathogenetic lesions following vagotomy, at one-half to six hours after an operative procedure. Pelikan ink is injected into the tail vein 5 minutes before sacrifice of each animal. This may afford a demonstration of the intimate vascular site in the lung where plasma leakage first occurs. Complete series of animals have been prepared for examination by light and electron microscopy. In addition, lung surface extracts of vagotiomized animals have been tudied on the Wilheimy balance, and specimens of lungs showing altered e tract have been prepared by the method of Laznitzky to observe whether the normal alveolar membrane d1sappears at the time that surfactant is altered. Detailed information from this materiial will be available in few weeks; preliminary micrographs suggest that, following vagotomy, both the vascular endothelium and the alveolar epithelium may be simultaneously involved. Microvilli disappear from the air surface of alveolar cells, so that the cells resemble macrophagies in the septal spaces. Membranous alveolar epithellum is thickened, and an active inflammatory response proceeds in the pulmonary capillaries. Cursory examination of the ink-injected lungs indicates that the leakage appears at the capillary level, but this requires much more study.

III. Anti enicit of dioalmltyl lecithin (DPL) in rabbits.
(Craig, Buckingham, Rothenberg, McNary)

Craig has localized fluorescent-tagged antibody to washed human lung foam (DPL containing protein) in human lungs. He indicates that, in fetal lung, antibody appears in the differentiating alveolar cell before the time it Is observed at the alveolar interface as the normal lung lining layer. Of great interest is his finding that the abnormal alveoiar hyaline membranes of infant lungs contain huge amounts of fluorescent-tagged material indicating, as previously suggested (3), that bronchiolar and alveolar cell necroblosis was a prominent part of the infant disease in which contents of epithelial cell were incorporated into the membrane. Craig further suggests that lung lining layer already secreted may find its way into the membrane, thus accounting for the continuing progressive atelectasis that appears to characterize this illness. (34)

We discussed his antigen with Craig in terms of its non-specificity (obtained as it was from lung foam which, despite many washings, contains various lung proteins, some probably derived even from plasma). It was our hope that he might obtain even more significant data if an antigen, resembling the surface active moiety of lung foam, were used to stimulate antibody production. We first thought of trying dipalmityl lecithin, now available in synthetic form. This has been mixed with Freund's adjuvant and injected in rabbits at weekly intervals x4'. Trial bleeding ten days after the last injection has failed so far to demonstrate any antibody, as measured on an Ouchterlony plate. The rabbits are being rested, and a series of antigen injections will be reinstituted for six weeks more.

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A trial of antibody formation will also be tested in sheep (whose antibody production is far superior to other mammals), using this trial antigen as a first approximation. Recovery of any antibody will also facilitate the study of the localization of ferritin labeled antibody in alveolar cells by electron microscopy (McNary).

If this phosphatide is not antigenic, we will discuss with an organic chemist the possibility of conjugating the DPL molecule to increase its antigenicity.

Craig's data are so interesting, it is too bad that the non-specificity of his antigen may vitiate his elegant findings.

IV. Entodermal origin of large pulmonary alveolar cells. (Volkmann and Buckingham)

Neither the site nor the germ layer of origin of the large alveolar (septal) cells is certain.(35) All the evidence obtained to date in our laboratory in re the morphologic differentiation of the fetal alveolar elements suggests an epithelial cell origin. Early in development, the epithelial cells resemble each other, later taking on morphologic characteristics of septal and membranous cells. Furthermore, we feel that the "lung-like" phospholipid synthetic activity that we have now localized specifically to the large alveolar cell (1) indirectly speaks to its origin, putting it at least in the lung. This makes it unlikely that even if the cell is mesodermal its precursors arise from bone marrow, as do peritoneal macrophages. (36)

These studies will use C-3 H-black inbred mice (that can tolerate parabiosis). We have induced the multiplication of pulmonary alveolar wall cells and macrophages by intravenous administration of BCG, noting the increase in the cells of the alveolus 24 and 48 hours after injection. We will study the latent period of recovery of tritiated thymidine label in the harvest of alveolar cells and in lungs of animals sacrificed for periods up to 12 to 16 hours after I.V. label injection. Considerable appearance of label at this time diminishes the possibility of a distant mesodermal origin. This can be made more convincing in parabionts.

These experiments will be to the stage of thymidine injection by February 1966. The survival of parabionts is a technically limiting factor at the present time.

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V. Manufacture of acid mucopolysaccharide in large alveolar cell inclusions. (Buckingham and McNary)

We think it is possible and relevant to understanding the integrity of the alveolar membrane that mucoid material underlies the surface active phospholipid in the pulmonary alveolar lining layer. This would correspond with Macklin's original suggestion. (37) Confirmation of the layer's PAS-positive, diastase-resistant staining properties at electron microscopic magnifications(38) further strengthens Macklin's claim. We have found that some cytoplasmic inclusions in the alveolar cells contain PAS-positive, diastase-resistant material. (1) (2) Interest in the nature and function of the surface active material, which we know to be phospholipid, has apparently diminished curiosity about what else is in the membrane. Its estimated thickness (200 to 3,000 Angstrom units) suggests a complex substance, and lipid + protein is not PAS-positive. Therefore, Macklin as usual is probably right. He supposed the function of this layer -- which he termed 'mucoid' -- was to preserve the interface from desiccation. One might also imagine that penetration of microörganisms through a jelly-like substance would make alveolar cell invasion and entry into the blood stream somewhat more difficult. Therefore, the possible role of the hydrated mucoid lining in the layer is certainly relevant to the biology of the alveolus. One could speculate that if such a layer altered its sol-gel state, as does connective tissue ground substance, great differences in permeability of the alveolar membrane might result.

What would be the nature of the mucoid? Since hyaluonic acid is non-sulfated, it does not take a PAS-positive, diastase-resistant stain. One is left with the probability of a sulfated mucoid, such as heparin or mucoitin sulphuric acid.

Screening experiments will study the radioautographic localization of S-35 in mice during a 24-hr. period. Localization in alveolar cell or macrophage cytoplasm and the time that this occurs can be estimated sufficiently that, in further experiments, electron microscopic radioautographs can be prepared by the method of Hay. Localization will be studied in comparison to that in proximal bronchiolar epithelium, as well as in the small intestinal epithelial cells of mice; these other tissues serving as controls.

VI. Precocious induction of fetal surfactant function. (Buckingham, Adamson, and McNary)

Since outlining this project in last year's grant application, we had an opportunity to discuss the project with Dr. Florence Moog, the zoölogist who discovered gut epithelium in developing the relationship between alkaline phosphatase activity and functional differentiation. (39) She encouraged us to prosecute the analogy in lung with experimental evidence. Fetal lung and fetal gut may be analogous tissues (both developing from entoderm), and may therefore be susceptible to some of these same fetal influences. Moog found that glycogen depletion and alkaline phosphatase enzyme activity increase heralded the morphological and functional differentiation of fetal gut; furthermore, the activity could be precociously induced by means of ACTH in some but not all species.

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In differentiating fetal pulmonary alveolar epithelium, glycogen storage is also abundant (2) (40) until just prior to the time that surfactant is first measurable in fetal lung; moreover, the inclusions that we rightly or wrongly associate with surfactant formation are alkaline phosphatase positive (2). Therefore, it is reasonable to wonder whether differentiation can be hastened, and surfactant production set earlier in gestation, by the administration of steroids. Dr. Karlis Adamson, Associate Professor of Obstetrics, will be the operator in these experiments. Fetal rabbits will be obtained well ahead of the period of gestation when surfactant normally develops in this species (term - 3 days). The maternal abdominal cavity will be opened surgically, and the amniotic sac of individual fetuses in one horn will be injected with ACTH; the fetuses in the opposite horn will serve as operative shams and will receive an equal volume of vehicle. The abdominal incision will be closed, the mother will be permitted to recover, and the fetuses will be delivered 24 to 48 hours later by caesarian section. At that time, the lungs of the treated fetal animals will be studied in two ways: 1) to determine whether surface activity is precociously present in ACTH-injected fetuses compared with controls; 2) histological and histochemical studies of the maturing fetal lung will assess the activity of alkaline phosphatase enzyme, the presence of glycogen, and morphological differentiation. Alkaline phosphatase will be studied at the magnification of the electron microscope.

These experiments will be carried out as soon as Dr. Adamson feels comfortable with the rather tricky technique of injecting amniotic sacs without subsequent leakage of amniotic fluid.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0008

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6. Budget Plan: †

† See complete budget sheet

a.	Salaries	$11,445
b.	Expendable Supplies	11,950
c.	Other Expenses	3,800
d.	Permanent Equipment	350
e.	Overhead (15% of a, b, c)	4,079
	Total	$31,624

7. Anticipated Duration of Work:

3-5 years

8. Facilities and Staff Available: My laboratory has moved to new space in the William Black Research Building, where facilities for animal experimentation, including radioactive studies, are self-contained within the Department of Pediatrics (4th floor) research unit. Drs. Sommers and Goldenberg offer a completely equipped Department of Pathology at Francis Delafield Hospital, across the street. There are two assistants who both work on two interrelated research projects (vide infra). Mrs. Mildred Rothenberg is in charge of lab business in addition to her duties as senior technician. Mr. King, a junior technician, runs the surface balance and, in addition, is assistant in all animal experiments. He is gaining some chemical expertise under guidance.

9. Additional Requirements:

None contemplated

10. Additional Information (Including relation of work to other projects and other sources of support):
My own salary and that of my senior technician are made available through an NIH research grant entitled Amine Handling as a Factor in Newborn Adaptation (HD-00746-), presently beginning its fourth year of support. The motivation for the two projects was a single idea and, as data presently reveal (section 1 and 2), the projects are inextricably associated. The 1966 total NIH support (part of which goes to Drs. Sommers and McNary) for 1966 is $39,000. I plan to continue separate investigations under an NIH grant unrelated to lung surfactant and, in 1968, will apply for a 5-year renewal of NIH support.

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Signature Dr Brough MD
Director of Project

W Stanton Harriss
Business Officer of the Institution W. Stanton Harriss, M.A.
Title Associate Director for Administrative Affairs

Source: https://www.industrydocuments.ucsf.edu/docs/qlwn0000

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	rand Tota l	31,624

r. S. C. Sommers.

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THE COUNCIL FOR TOBACCO RESEARCH U.S.A.

CONFIDENTIAL

February 14, 1966

MEMORANDUM

TO: Committee comprising Dr. Cattell, Chairman, Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: New grant application from Samuel Bellet, M.D. - #530.

We enclose herewith a new grant application from Dr. Samuel Bellet of the Philadelphia General Hospital for a study of the effects of caffeine on blood lipids.

This proposal at present is being sent only to members of the committee, but we plan to include it in the agenda for the March meeting along with a renewal application for a continuation of Dr. Bellet's work previously supported by CTR.

P.S. A reprint will be mailed later.

R. C. H.

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CARDIOVASCULAR, PHARMACOLOGY, CHEMISTRY

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE TOBACCO INDUSTRY RESEARCH COMMITTEE

633 THIRD AVENUE

NEW YORK, N. Y. 10017

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COMMITTEE

r. Cattell, Chm

Dr. Bing

r. Jacobson

pplication For Research Grant

at.: January 27, 1966

Name of Investigator: SAMUEL BELLET, M.D.

Title: Director, Division of Cardiology

Institution & Address: PHILADELPHIA GENERAL HOSPITAL
34th Street and Curie Avenue
Philadelphia, Pennsylvania 19104

Project or Subject: THE EFFECT OF CAFFEINE ON BLOOD LIPIDS

1. The Effect of Caffeine on Diabetics
2. The Effect of Caffeine on Urinary Catecholamines
3. The Effect of Caffeine & Smoking on Free Fatty Acids
4. The Effect of Caffeine on Blood Coagulation
5. Studies in the Experimental Animal
6. Effect of Chronic Administration of Caffeine on the Lipid Fractions in Chronic Experiments
7. Basic Levels of Lipids in Chronic Caffeine Subjects
8. The Effect of Caffeine on the Normal Subject

etail.d Plan of Procedure (Use additional pages if more spaa is rpuired.)

(SEE CONTINUATION SHEETS 01 - 3)

10

3

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$23,445.05
b.	Expendable Supplies	5,430.00
c.	Other Expenses	
d.	Permanent Equipment	4,331.26
e.	Overhead (15% of a, b, c)	
	Total	$33,206.31

7. Anticipated Duration of Work: THREE YEARS

8. Facilities and Staff Available:

SEE CONTINUATION SHEETS #5 - 6)

9. Additional Requirements:

NONE

10. Additional Information (Including relation of work to other projects and other sources of support):

NONE

Signature

Director of Project

Business Officer of the Institution

Benjamin F. Burgess, Jr.
Director of Research

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

5. DETAILED PLAN OF PROCEDURE:

1. Current Status of Work in Area of this Proposal: There is very little literature relative to the relationship between caffeine and its effects on blood lipids. There is also little data concerning the relationship of caffeine administration to the prevalence of atherosclerosis. The only experimental data we were able to find was that of Czochra-Lysanowicz and Mjasknikow where caffeine was observed to produce atherosclerosis in rabbits (6, 13).

Several epidemiologic studies have recently been performed suggesting a relationship between the effect of caffeinated beverages and coronary artery disease (14, 20).

Recently, work in our laboratory has shown that both caffeine and coffee significantly elevated the free fatty acid level (4). Moreover, it is fairly well recognized that a repeated increase in plasma free fatty acids may predispose to a rise in lipoprotein lipids (17). The administration of caffeine in certain respects bears a striking parallel to that of nicotine. The effects of nicotine and smoking have been studied fairly extensively in our laboratory (2, 3, 11, 12); the effects of caffeine on FFA (0.5 Gm) are quite similar to and somewhat more prolonged than that of smoking (2 cigarettes). Smoking in humans and nicotine in dogs caused a rapid and consistent rise in serum FFA levels and patients with myocardial infarction developed an elevation more than twice that of normal subjects and non-coronary patients. In dogs given nicotine daily for a 6-week period, there was a 50% rise in the level of serum cholesterol.

Some lipolytic effects of caffeine have been studied in vivo and in vitro, many of which appear to be opposite to those of insulin (7, 18). Like epinephrine (16, 19), caffeine potentiates cyclic 3'5' AMP and phosphorylase activity (5, 9, 10, 15) and produces both glycogenolysis (5, 8) and lipolysis (4, 7, 18).

2. Specific Aims: The specific aims are to study the relationship between caffeine and blood lipids in the human subject and in the experimental animal in the acute stage and following chronic administration. Specifically, the following will be studied: (a) The effect of caffeine in normal subjects (amplification of our previously published work). This will include adults in the age group between 17-25 and those between the ages of 40-65. (b) Studies will be performed in children, ages 4-16. There are some suggestions that children may handle caffeine somewhat differently from adults. (c) The effect of caffeine in diabetics. This would seem to be of some importance since caffeine manifests an influence in the carbohydrate metabolism and caffeine and insulin have been shown to be antagonists ( ). In addition, many diabetics are coffee drinkers. (d) The simultaneous effects of caffeine and smoking. Preliminary experiments have shown that caffeine modifies the effect of smoking in blood lipids. (e) The effects of concomitant administration of caffeine and glucose. (f) The effects of beta-blocking agents on the administration of caffeine in order to determine whether the FFA production is abolished in a manner similar to that of opinephrine and nicotine. (g) The effects on blood coagulation in the human subject (see below). (h) The study of the basic level of triglycerides and cholesterol in human subjects who have been drinking coffee for years.

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3. Methods of Procedure: Twenty-five normal subjects, aged 17-25, and 25 subjects, aged 40-65, will be studied in the fasting state. The subjects will be kept at rest and free of environmental stimulation and will be prohibited from smoking during the experiment. The following series of observations will be made on these groups. On one day, 5 Gm of soluble instant coffee powder will be ingested over a 15-minute period. In a second series of experiments, the patients will be given 500 cc. of hot water with 3 tablets of sweetener as a control. In the third series, caffeine sodium benzoate (0.5 Gm of each buffered in saline) will be given intramuscularly. Blood samples taken from an atencubital vein will be taken before, 30,60,90,120 and 180 minutes after the ingestion of the beverage. Since our previous experiments have shown that the effects on FFA extends over 180 minutes, we will extend our period of administration(on some patients) after caffeine administration to 4 hours. Serum FFA concentrations will be determined in all experiments. Serum triglycerides, cholesterol and plasma glucose will also be determined.

Similar studies will be performed in children who will be given caffeine in doses of 8-10 mg/Kg.

Studies similar to the normal group will be repeated in patients with coronary artery disease; included will be subjects with previous myocardial infarction. Studies of this group should be of interest since it has been shown that these subjects have increased sympathetic tone and show an increase in circulating catecholamines with higher control levels of FFA. Statistical significance of the data obtained will be determined by means of the t-test.

Studies of Diabetics: Patients will come to the Hospital at 8:00 a.m., having had nothing to eat or drink since midnight and no insulin for 24 hours. Blood will be drawn for blood sugar and plasma FFA. 500 mg of caffeine sodium benzoate will be administered parenterally. Blood will be drawn for blood glucose and plasma FFA at 30, 60, 90, 120 and 180 minutes after the administration of caffeine sodium benzoate. Subsequently, the same patients will be studied in a similar manner but without receiving the caffeine sodium benzoate.

Studies on Urinary Catecholamines: Ten experiments will be performed in which the urine will be studied for catecholamines during a control period of 4--6 hours and for a similar period after caffeine has been administered. The purpose of this study is to define the effect of caffeine administration on urinary catecholamines and to compare these values with those of other preparations that produce an increase in FFA, e.g., epinephrine and nicotine (2).

Effect of Caffeine and Smoking on Free Fatty Acids: This will be determined in subjects where the effects of caffeine and nicotine on separate days have been observed and the effects on FFA noted. The combination of these two will then be studied. Preliminary studies have indicated that the rise in FFA, instead of being additive, is much less marked.

Blood Coagulation Studies: We propose to study the coagulation profile of caffeine in the human subject and in the experimental animal after the administration of 2½ cups of coffee (containing 218 mg of caffeine) and after 500 mg of caffeine. The following clotting studies will be performed. (1) The clotting time in glass and silicone; (2) Plasma studies consisting of (a) One-stage prothrombin thrombin time of plasma, (b) Stypzen time, (c) Plasmia fibrinogen concentrations, (d) Recalcification time of the plasma, (e) Heparin tolerance times, and (f) Thromboelastography; and (3) Fibrinolytic studies consisting of (a) Euglobulin lysis times, (b) Zones of lysis of fibrin plates, and (c) Urokinase lysis times.

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Studies in the Experimental Animal: The following series of acute experiments will be performed on dogs involving the administration of caffeine in a dose of 20-25 mg/Kg body weight. Control samples will be taken before and at intervals of ½ hour to 4 hours. These will be studied for FFA. Preliminary experiments have shown a uniform rise of FFA with these experiments. The effects of methoxamine, hexamethonum, reserpine, Inderal, C-60 will be studied. The purpose will be to determine the degree of blocking effects on FFA production by the various drugs administered.

The Effect of Chronic Administration of Caffeine on the Lipid Fractions in Chronic Experiments: At the present time we have been studying a series of dogs in whom caffeine has been administered daily for a period of 6 weeks. It is too early to determine significant effects. We plan to utilize the rabbit, rat and squirrel monkey for such chronic experiments. We feel that the result should be of some interest.

Basic Levels of Lipids in Chronic Caffeine Subjects: It is proposed to determine the levels of triglycerides and cholesterol in a group of 200 subjects between the ages of 40-65 who have been drinking 4-6 cups of coffee/day over a period of years. This data will be compared to a similar group of non-coffee drinkers (or those who drink only 1-2 cups/day).

3. Significance of this Research: As stated above, the possible connection between caffeine and coronary heart disease is of primary importance. Keys to the mechanism of FFA response and lipoprotein-lipid increments are important steps in this direction. Because coffee is one of the most widely consumed beverages in the United States, data relating to the physiologic effects of caffeine is highly significant. In general, these findings will be of interest in view of the suggested relationship of FFA elevation to increments in other lipid fractions, notably, triglycerides and cholesterol.

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BUDGET PLAN:

a. Salaries

Principal Investigator	10%	$ 2,000.00
Biochemist III	50%	5,190.00
Medical Laboratory Technician II	100%	5,389.00
Secretary (Clerk-Steno III)	50%	2,694.00
Fellow in Cardiology	100%	6,000.00
Pension (5.7%)		1,212.56
Workmen's Compensation ($.31 per $100)		66.03
Social Security		893.46
		$ 23,445.05

b. Consumable Supplies

Dogs 40 @ $15.	600.00
Vaccines and Medicine @$1.50 each	60.00
Maintenance @ $.50 per dog per day	2,500.00
Rats 200 @$1.25	250.00
Maintenance for 16 weeks	320.00
Glassware	500.00
Chemicals	1,200.00
	$ 5,430.00

c. Permanent Equipment

NONE

d. Overhead (15% of a and b)	$ 4,331.26
GRAND TOTAL	$ 33,206.31

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ACILITIES AND STAFF AVAILABLE:

2) General Facilities--

, A 2,500 bed municipally owned and operated general ospitall staffed by the faculties of 5 medical schools, both full-time and part-time. A large percentage of the beds are used for cardiovascular cases. pecialty clinics which are taffed and used by the Division of Cardiology include an adult and pediatric heart clinic, hypertension clinic, peripheral vascular clinic and diabetic clinic among others. The ivision of Cardiology is responsible for all problems relating o the subject of cardiovascular disease for the entire hospital.

. The Division of Cardiology Consists of:

. A Heart Station -equipped with complete instrumentation for ardiovascular diagnosis: Electrocardiography, Radioelectro- cardiography, Phonocardiography, Vectorcardiography, Ballisto- ardiography, Fluoroscopy (Image Intensifier), Cardiac Catheter- zation, Angiography, Peripheral Vascular Diagnosis (Constant emperature Room), a Library and a Reading Room.

. A Biochemical Laboratory devoted fully to cardiovascular esearch (distinct from the hospital clinical laboratory). It is equipped for doing lipid analysis, catecholamine analysis, lood gas analysis, flame photometry, electrophoresis, osmometry, pectrophotometry, colorimetry, chromatography, polarography, etc.

. An Isotope Laboratory for radioactive tracer studies

n Animal Laboratory

, An Enzyme and Drug Evaluation Laboratory

6, A Pulmonary Function Section

Major Items of Permanent Equipment (Biochemical Laboratory):

. Well-type scintillation counter, scaler, probe, rate meter, and pulse height analyzer (NRD).

. A vibrating reed electrometer (Nuclear-Chicago) with lide chamber, glassware, ionization chambers for measuring weak Beta radioactivity of Tritium, Carbon-14,, and Sulphur-35, in olid,, liquid and gaseous phase.

, Ultracentrifuge

. Refrigerator centrifuge

. Fraction collector

. Column chromatography equipment

. Gas chromatography equipment:

. Paper electrophoresis equipment

ensitometer (Photovolt)

pectrophotometer (Beckman)

, Fluorimeter (Farrand)

1. Densitometer (Photovolt)

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m. Colorimeters (Evelyn-Klett)
n. Osmometer (Fiske)
o. Van Slyke volumetric & manometric apparatus
p. Scholander respired air analyzer
q. pH meters (Beckman Model G & Metrohm)
r. Polarograph
s. Respirometer
t. Dual count rate meter, recorder and scintillation counter combination.

3. Staff Available:

Director of the Division of Cardiology
Section Chief: Section of General Biochemistry
Section of Radioisotope Studies
Section of Adult Hemodynamics.
Section of Pediatric Hemodynamics
Section of Peripheral Vascular Disease
Section of Drug Biochemical Laboratory
Section of Animal Research
Clinical Section
Electronics Section

5 Attending Physicians
1 Electronic Engineer
10 Residents & Fellows in Cardiology
2 Technicians
5 Laboratory Technicians

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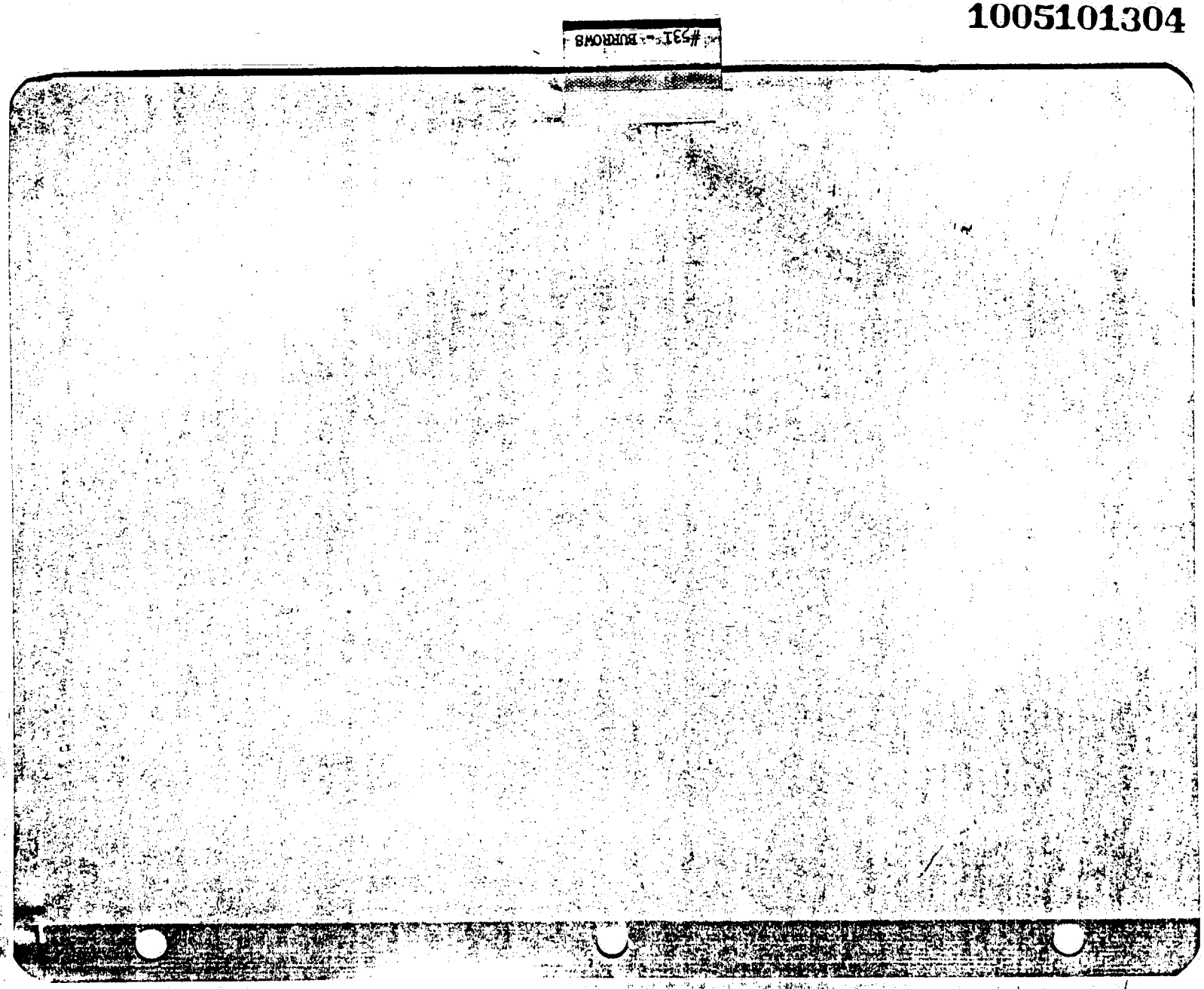

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~ COUNCIL FOR TOBACCO RESEARCH-U.S.A. ~ CONFIDENT Febr uary 14, 1966

MEMORANDUM

W: The committee comprising Dr. Jacobson, Chm., Dr. Bing and Dr. Lynch

FROMt Robert C. Hockett

UBJECT: Application from Benjamin Burrows, M.D. - No. 531

We enclose herewith an application from Dr. Benjamin Burrows of the University of Chicago, for support of a research project related to the one which we have supported for the past two years (No. 345).

A progress report for work done under the previous grant has not been eceived, but Dr. Burrows writes that a manuscript is in preparation.

This application is being sent at present only to members of the committee, but we plan to include it in the agenda for the March meeting.

C. H.

530

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

PULMONARY PHYSIOLOGY

#531

Cr. #408
Activated: 7/1/64
Renewed: 7/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Bing
Dr. Lynch

Application For Research Grant

Date: JANUARY 17, 1966

1. Name of Investigator: BENJAMIN BURROWS, M.D.

2. Title: Associate Professor of Medicine

3. Institution & Address: UNIVERSITY OF CHICAGO
950 East 59th Street
Chicago, Illinois 60637

4. Project or Subject: A CORRELATION OF SEVERITY OF SYMPTOMS OF CHRONIC OBSTRUCTIVE LUNG DISEASE WITH ENVIRONMENTAL FACTORS.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

For the past several years, we have collected daily diary information on a group of patients with chronic obstructive lung disease (the asthma-bronchitis syndrome). This information has been processed for high speed electronic computation. Detailed meteorological data, considerable air pollution information and pollen counts for two of the years has been made available to us by an agency of the City of Chicago, and this material is being processed into an electronic computer. It is planned to correlate severity of symptoms with climatic, air pollution, and pollen data.

First, the pattern of exacerbation of symptoms must be fully documented, including intercorrelation of individual symptoms, in order to properly scale each day as to severity of symptoms and identify days in which exacerbations are likely to begin.

Second, meterological data must be reduced to daily observations. This involves such problems as averaging temperatures, determining hours of certain wind patterns, etc.

Third, certain characteristic weather patterns must be identified and scaled.

Finally, the derived information must be correlated using multiple regression techniques in order to determine which environmental factors appear most important in determining severity of symptoms.

The entire project involves extensive computation and is time consuming because of both the extent and complexity of the data. (At present, daily diary information is on approximately 15,000 and environmental data on over 53,000 IBM cards).

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6 Budget Plan:

a.	Salaries (Research Asst. & Programming)	8,000
b.	Expendable Supplies	200
c.	Other Expenses (Travel & Computer time)	800
d.	Permanent Equipment	
e.	Overhead (15% of a, b, c)	1,350
	Total	10,350

7. Anticipated Duration of Work: Two years

8. Facilities and Staff Available: The facilities of the Biological Sciences Computation Center with 1401 and 7094 computers plus statistical and programming consultants. Offices and laboratories of the investigator including clinical and technical personnel, physiology laboratories, desk calculator, etc.

9. Additional Requirements: As noted, the data to be analyzed has already been collected and partially processed for electronic computation. However, a great deal of work remains to be done before any meaningful interpretation of the data can be made. The major requirement is for a Research Assistant who can handle the computer programming and assist in checking and interpretating results.

10. Additional Information (Including relation of work to otherprojects and other sources of support):

This project is closely related to the major research effort of this investigator, namely a Public Health Service supported study on the natural history of the "emphysema-bronchitis syndrome." Equipment and support for clinical aspects of the study come from Public Health funds. The present research represents a diversion from the main project and requires additional personnel plus ainmall amount of ancillary support.

This research is of considerable importance, for/delineating environmental factors which tend to exacerbate the "emphysema-bronchitis syndrome", one may be able to improve the therapy of this disorder and perhaps draw inferences concerning its pathogenesis.

Signature
Director of Project

Business Officer of the Institution
M. T. Tracht, Business Administrator
Division of Biological Sciences

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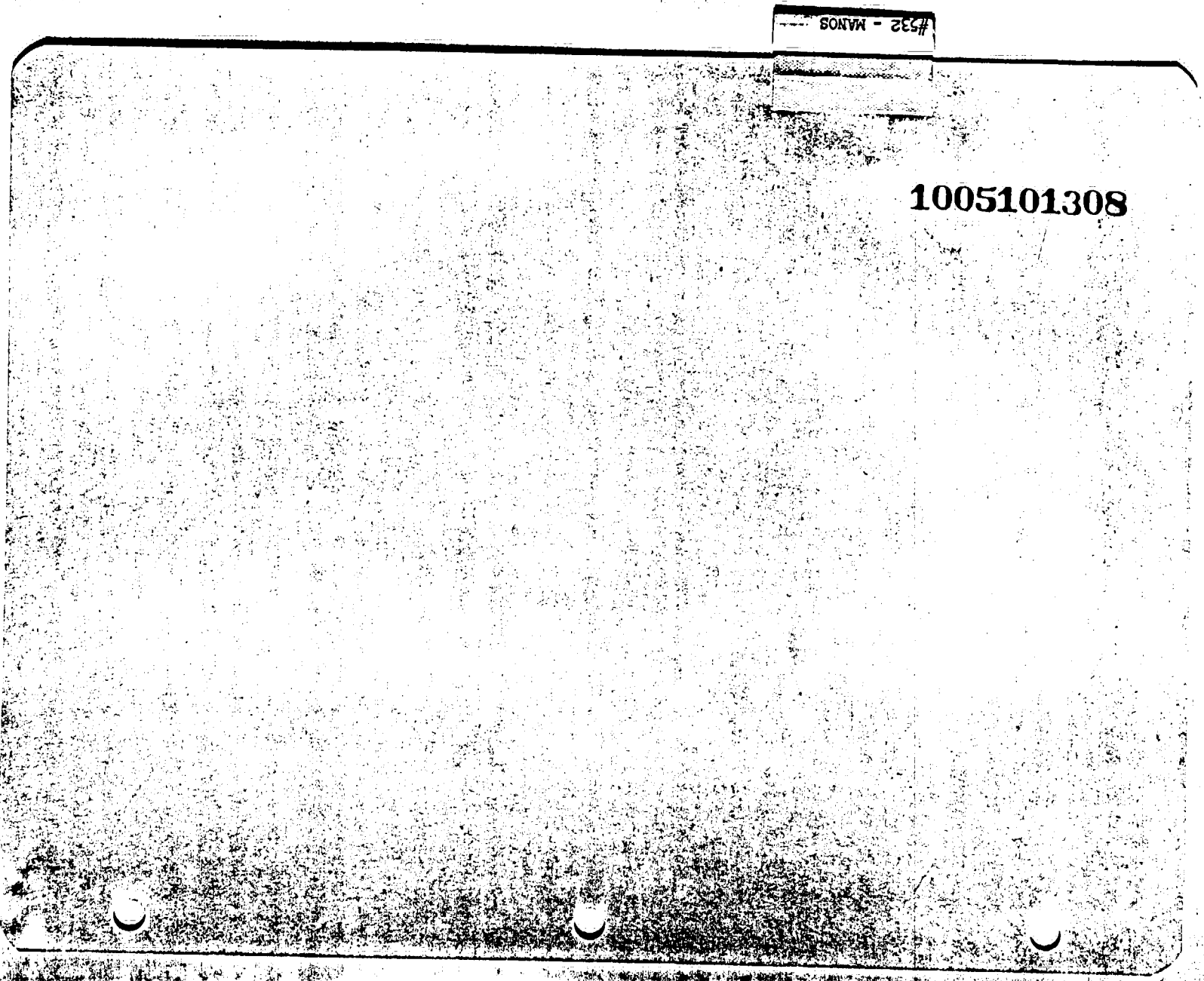

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MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chairman, Dr. Iynch and Dr. Little.

FROM: Robert C. Rockett

SUBJECT: New grant application of John P. Manos, M.D. - #5312.

Dr. ..

We enclose herewith a new grant application from John P. Manos of the Medical College of South Carolina.

This application at present is being sent only to embers of the committee, but we plan to include it in the genda of the March meeting along with a renewal application or a closely related project presently supported by The Council..

C.H.

CH:ek

ncl.

13

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

VIRUS, Previous Infection

#532
Cf. #362

Activated: 5/1/63
Renewed: 5/1/64
Renewed: 5/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Lynch
Dr. Little

Application For Research Grant

Date: January 31, 1966

1. Name of Investigator: JOHN P. MANOS, M.D.

2. Title: Associate in Microbiology

3. Institution & Address: MEDICAL COLLEGE OF SOUTH CAROLINA
80 Barre Street
Charleston, South Carolina 29401

4. Project or Subject: THE ANTIGENIC STUDY OF THE VARIOUS NEOPLASTIC SUBCELLULAR PARTICLES FOLLOWING SEPARATION BY ULTRA-FILTRATION METHODS.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

That neoplastic cells hava an antigenic makeup distinct at least in part from normal cells has been a source of much interest and investigation. The significance of such studies for potential diagnostic, therapeutic, preventive and etiologic evaluations is obvious. Some pertinent references are listed.

As an outgrowth of a project currently in progress, it was felt that an antigenic study of subcellular fractions of neoplastic cells by methods used in the other project, i.e. differential ultra-filtration, the emergence of specific 'cancer' antigens might be effected. As indicated in progress report #4, work has been begun in this area. Cells from a human lung cancer and normal human lung have been disrupted mechanically by grinding and the sub-cellular material fractionated by ultra-filtration methods using the Gelman filterfuge apparatus.

These fractions, 10 from the human lung carcinoma and 10 from normal human lung, have been inoculated in paired rabbits using Freund's complete adjuvant and an intensive immunization schedule. The rabbits were exsanguinated and the cell fractions are being tested for antigenicity. The first method of approach is that of gel double immunodiffusion. Preliminary work by this method has shown that some of the fractions so far tested, both from cancer and normal lung cell are antigenic. It is planned, by cross absorption techniques to determine if any of the cancer cell fractions have or lack antigenic component(s) absent or present in the normal cell fractions. Other methods to be used for determining antigen specificity are the tanned red cell and possibly the complement-fixation techniques. Also, other approaches to the preparation of fractions could be used, such as density gradient centrifugation and column chromatography.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	-
b.	Expendable Supplies	5000.00
c.	Other Expenses	1000.00
d.	Permanent Equipment	3000.00
e.	Overhead (15% of a, b, c)	900.00
	Total	9900.00

7. Anticipated Duration of Work:

3 Years

8. Facilities and Staff Available:

Millipore filter apparatus and filters, Gelman centrifuge filter holders and filter, various centrifuges, including the Serval Regrigerated super-speed centrifuge, spectronhotometers, Micro Kjeldahl equipment, photographic equipment, chromatographic apparatus, sonic oscillators, fluorescent microscopy equipment, electron microscopy and other equipment pertinent to microbiological work. Apparatus for double immunodiffusion techniques and other serological techniques. Column chromatographic apparatus.

9. Additional Requirements: Need for a preparative ultra-centrifuge in the future.

10. Additional Information (Including relation of work to other projects and other sources of support):

The methods used for subcellular fraction preparation derived from current project studying similar type fractions from mouse lung adenomas in an attempt to demonstrate viral activity in mice and tissue cultures. This latter project is presently being supported by The Council For Tobacco Research-U.S.A.

Signature John C. Manos, M.D.
Director of Project

[signature]
Business Officer of the Institution

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#533 - FORST

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. CONFI

ebruary 16, 1966

EMORANDUM

TO: The committee comprising Dr. Jacobson, Chairman, Dr. Andervont, Dr. Reimann and Dr. Little.

ROM: Robert C. Hockett

UBJECT: New grant application from Arthur Furst, Ph.D. - #533.

We enclose herewith a new grant application from Dr. Arthur urst of the University of SanFrancisco.

t proposes a one-year extension of the previous three-year tudy, which was activated on August 1, 1962 (#349) and has been ompleted. A progress report summarizing the results of this previous tudy is attached.

site visit to this project was made by R.C.H. on ebruary 2, 1966. Furst's Institute of Chemical Biology is now housed n a fine new building at the University of San Francisco and his facilities re excellent.

he application presented here was instigated by the CTR taff and its plan evolved by conference, with the purpose of trying to "wrap up" the question of nickel in tobacco as a possible factor in lung ancer insofar as the bioassay used in these experiments makes this ossible. Furst will be given the best available information on smoke nhalation techniques as improved by other CTR projects.

.C.H.

CH:e

ncl.

ersonal Opinion: Dr. Furst seems to me to be one of our most competent nvestigators in this area, and I not only favor this rant but feel that his facilities can be applied to other very pertinent problems in the future.

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BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

#533
Cf. #349
Activated: 8/1/62
Renewed: 8/1/63
Renewed: 8/1/64
Extended to 1/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.
SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Andervont
Dr. Reimann
Dr. Little

Application For Research Grant

Date: February 9, 1966

1. Name of Investigator: ARTHUR FURST, PH.D.

2. Title: Director, Institute of Chemical Biology

3. Institution & Address: UNIVERSITY OF SAN FRANCISCO
San Francisco, California 94117

4. Project or Subject: NICKEL IN RELATION TO THE SMOKING AND LUNG CANCER PROBLEM

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

5. Detailed Plan of Procedure:

Nickel, which is found in cigarettes, has been implicated as a causative agent in the smoking-lung cancer controversy. Many compounds of nickel are carcinogenic. Sunderman claims that nickel carbonyl is carcinogenic (Am. J. Clin. Path., 35, 203 (1961)); Clayson, D.B., in his book "Chemical Carcinogenesis" (Boston, Little, Brown & Co., 1962), objects to this conclusion for only nine rats including controls survived in Sunderman's experiment.

Hueper induced tumors at the site by parenterally administered metallic nickel or some nickel salts. His work is summarized in "Chemical Carcinogenesis and Cancers",

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

by Hueper, W. C., and Conway, W. D. (Springfield, Ill., Charles C. Thomas Co., 1964).

Furst (unpublished work) also induced tumors in rats by IM injections of either nickel powder or organometallic nickel compounds suspended in a synthetic triglyceride trioctanoin medium. Furst also studied the effect of adding quantities of nickel (1000X) to cigarettes, and exposing mice to the smoke of these nickel-enriched cigarettes. A summary of the last project is enclosed. (See appended Progress Report.)

Projects Proposed:

A one-year project is proposed to study three phases of this problem.

I - Effect of Nickel-Enriched Cigarettes on the Induction of Pulmonary Adenomas in Mice

We have shown that by exposing mice to smoke from cigarettes enriched with nickel acetate (1000X the normal nickel content) it is possible to induce tumors. At what level does this phenomenon disappear? We will repeat our experiments using less nickel per cigarette, in each group. Following the usual procedures of pharmacologists, we will decrease the nickel content geometrically. Thus we will repeat our work using values of 500 mg./cigarette, 250, and 125 mg./cigarette... Ten mice per level will be used at any one time and the experiments will be repeated 10 times, giving a total of 100 mice at any one level. Using these

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data, we will plot: total tumors/level vs. level of nickel/cigarette.

From the graph we can determine at what level the nickel is insignificant, and thus if the nickel contained in the cigarette can be considered harmful.

I - Effect of Nickel Carbonyl on the Induction of Lung Tumors in Mice

Since Sunderman postulated that the nickel in the igarette is converted to nickel carbonyl (Ni(C0)4) during he process of smoking and that this compound is the carcino- en, we will study the potential carcinogenic effect of i((CO)4 on mice. Mice will be exposed to various concentra- ions of nickel carbonyl. Because of the very low levels f this volatile liquid which must be used, we will augment ur experiments with mice injected with subcarcinogenic evels of urethan. In the usual urethan experiments, animals re given 1 mg./gm. of body weight. In these experiments e will use 0.08 mg./gm., a value which has been found to e inactive. A combination of Ni(C0)4 and subcarcinogenic evels of urethan will be used. We already have data for LD50 f Ni(C0)4 and for the rate of appearance of tumors after rethan administration.

e have also developed an ultramicro quantitative deter- imation of nickel. We will use this method to see if the nimal xcretes nickel after exposure to nickel carbonyl.

II - The third phase will be an attempt to determine the ature of the nickel already present in the cigarette. e wil'l also attempt to identify nickel carbonyl in igarette smoke by the gas chromatographic method. amples from raw tobacco and manufactured cigarettes will e used.

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BUDGET (One Year)

Personnel:

Principal Investigator - A. Furst		
Biologist, M.S. (equiv.) - 100%	$650x12	$ 7,800
Laboratory Assistant, Part-time	$250x12	3,000
Animal Helper, Part-time	$175x12	2,100
Subtotal		$12,900
University required fringe benefits, 12%		1,548
Total Personnel		$14,448

Supplies:

Animals	1,000
Chemicals	800

Travel:

One trip to East Coast	450
Partial payment - International Cancer Congress, Tokyo	400
	$17,098

Overhead:

University usually requires 30% overhead; this project approved at 15%.	2,565
TOTAL	$19,663

Signatures:

Director of Project — Arthur Furst
Arthur Furst, Ph.D.

Business Officer — James M. Corbett S.J.
James M. Corbett, S.J.
Treasurer

University Official — Edmond J. Smyth S.J.
Edmond J. Smyth, S.J.
Dean, College of Science

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#536 - HADDY

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HE COUNCIL FOR TOBACCO RESEARCH — U.S.A.

February 18, 1966

MEMORANDUM

TO: The committee comprising Dr. Bing, Chm., Dr. Cattell and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: New grant applicatibn from Francis J. Haddy, M.D., Ph.D. - No. 536.

We enclose herewith, a new grant application from Dr. Francis J. Haddy, ow of the University of Oklahoma Medical Center, who will become Chairman of the epartment of ftsiology at Michigan State University on July 1, 1966.

r. Haddy receivedia previous grant (#433) which was activated on uly 1, 1964 and renewed on July 1, 965: to coaplete the two-year plan he proposed riginally. These grants were for $31l,010 and $23,270 respectively. Our original commitment has thus been completed and no projection has therefore been made for ur 1966-67 budget. The present proposal is therefore construed as a new one.

progress report for the calendar year 1965 is enclosed.

This application is being distributed at present only to members of he committee but we plan to include it on the agenda for March 5-6.

. C. H.

Personal Opinion: No visit to this project has been made by staff or board embers during its two years and no evaluation of the smoke roduction techniiques carried out. The reference to use of. ilter cigarettes is a bit disturbing. It seems to me that conference and general evaluation review should be con- ted before any new grant is made. Action at the May meeting ould still anticipate the July lst anniversary of the project. ence,, I would recommend deferring action until May, with ar- angements for a staff or committee visit, or tentative action ontingent upon the recommendations of a review committee.

. C. H.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
630 THIRD AVENUE
NEW YORK, N. Y. 10017

No. 536
Cf. #433
Activated: 7/1/64
Renewed: 7/1/65

COMMITTEE:
Dr. Bing, Chm.
Dr. Cattell
Dr. Jacobson

Application For Research Grant

Date: January 31, 1966

1. Name of Investigator: Francis J. Haddy, M.D., Ph.D.

2. Title: Professor and Chairman
Department of Physiology

3. Institution & Address: Michigan State University
Giltner Hall
East Lansing, Michigan

4. Project or Subject: Acute Local Effects of Inhalation of Tobacco Smoke on the Cardiovascular System

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

The effects of smoking on heart rate, blood pressure and peripheral blood flow are documented. The mechanisms of the effects are, however, not well understood. Do the effects on blood flow, for example, result from a direct action of the products of tobacco smoke on blood vessels as well as indirectly via release of vasoactive substances from the adrenal medulla, juxtaglomerular cells of the kidney, etc.? During the first year of the project, we studied the direct effect of inhalation of filtered cigarette smoke on the total resistance to blood flow through the kidney, limb, and coronary vascular beds (see progress report no. 1). There appeared to be no direct effect on the limb and coronary beds and only a slight direct effect on the kidney. The responses seen seemed to result indirectly from release of catecholamines into the blood, possibly from the adrenal medulla. During the first half of the second year, we extended our studies to non-filtered cigarettes(see progress report no. 2). The findings in the limb were not different from those with filtered smoke. Thus the responses of the limb resulted from an indirect rather than a direct action of the smoke. We also examined the effect of non-filtered smoke on segmental resistances in the limb in order to make inferences about effects on arteries, small vessels and veins. We failed to show a direct action of non-filtered smoke on any one of these three types of vessels but noted that the small vessels and veins responded to the indirect action. Finally we examined the direct effects on limb total resistance of changes in the oxygen and carbon dioxide content of the inspired air and concluded that such changes, if induced by the burning cigarette, could have a significant effect during a fast local smoking.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

We now propose to 1). complete the studies outlined in our last proposal, 2) initiate new studies concerning the mechanism of the adrenal diischarge during smoking and 3) study the possible effects of smoking on the renin-angiotensin system.

1) Studies to be completed include the direct effects of non-filtered smoke on kidney, coronary, lung, brain and intestinal vessels. As before, an isolated lung will be interposed in the arterial supply of the organ and ventilated with air and non-filtered tobacco smoke. Blood flow to the organ under study will be held constant. Therefore changes in perfusion pressure will indicate changes in the resistance to blood flow. Since only the blood going to the organ will be exposed to the cigarette smoke, any response observed will result from a local action. When all of the blood is to be exposed to the cigarette smoke, the intact lung of the animal will be ventilated with air and cigarette smoke.

2) The studies of others as well as our own studies indicate a rise in the blood levels of catecholamines during smoking. This apparently results at least in part from an adrenal medullary discharge. The mechanism of this discharge not definitely known. For example, does the adrenal medulla discharge because f a direct effect of the products of tobacco smoke on the medullary cells or does it result indirectly through effects on the nervous system? We propose to study the direct effect of tobacco smoke on the adrenal medulla. The isolated lung will be interposed in the arterial supply of the adrenal and made to smoke a cigarette. lood pressure, heart rate and limb resistance will be monitored. Changes in these parameters will indicate a direct effect of cigarette smoke on the adrenal medulla since only the blbod going to the adrenal will be exposed to the smoke.

3) Could some of the pressure, resistance and flow changes observed during smoking result from activation of the renin mechanism? We propose to study the direct effect of tobacco smoke on renin release by interposing the lung in the renal artery, ventilating the lung with tobacco smoke, and measuring the pressor activity of renal venous blood. Arterial and renal venous blood will be collected in tubes containing EDTA to inactivate angiotensinase, the plasma incubated for 4 hours and then injected into the anesthetized, vagotomized, pentoliniumized rat according to the method of Page. Responses will be compared to an angiotensin standard. Since flow, arterial concentrations and venous concentration will be known, it will be possible to determine whether renin output per unit time is directly influenced by smoking.

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6. Budget Plan:

a.	Salaries	15,000
b.	Expendable Supplies	5,000
c.	Other Expenses	1,600
d.	Permanent Equipment	2,500
e.	Overhead (15% of a, b, c)	3,240
	Total	27,340

7. Anticipated Duration of Work: One to two years.

8. Facilities and Staff Available:

On July 1, 1966, the principal investigator will move from Oklahoma to Michigan State University as Chairman of the Department of Physiology. This department serves the new medical school as well as the school of veterinary medicine. This move will in no way slow the progress of the proposed research. On the contrary, it may well accelerate progress. All of the personnel are moving with the principal investigator and the facilities at Michigan State are superior to those in Oklahoma. One of three large well equipped cardiovascular laboratories in the Department of Physiology will be made available to the study. Certain common facilities such as animal quarters, shop, administrative offices, etc. will also be available to the study. The following investigators will be associated with the study:

F. J. Haddy, M.D., Ph.D., Professor and Chairman, Department of Physiology
J. M. Dabney, Ph.D., Associate Professor of Physiology
J. B. Scott, Ph.D., Associate Professor of Physiology
R. M. Daugherty, M.D., Ph.D., Associate Professor of Physiology

~~9. Additional Requirements:~~

~~10. Additional Information (Including relation of work to other projects and other sources of support):~~

9. Additional Requirements:

The budget changes reflect salary increments for a research associate (John Diana, Ph.D.) and a surgical technician. The permanent equipment category has been increased over the last year since several items (strain gauges, perfusion pumps) which are non-transferable from Oklahoma to Michigan State will have to be replaced.

10. Additional Information (Including relation of work to other projects and other sources of support):

The techniques necessary to this investigation have been perfected in our laboratories as a means of studying the mechanisms of local regulation of blood flow. These same techniques are ideally suited for the study of the effects of tobacco smoke on the cardiovascular system.

Signature Francis J. Haddy
Director of Project

Merrill R. Person
Business Officer of the Institution

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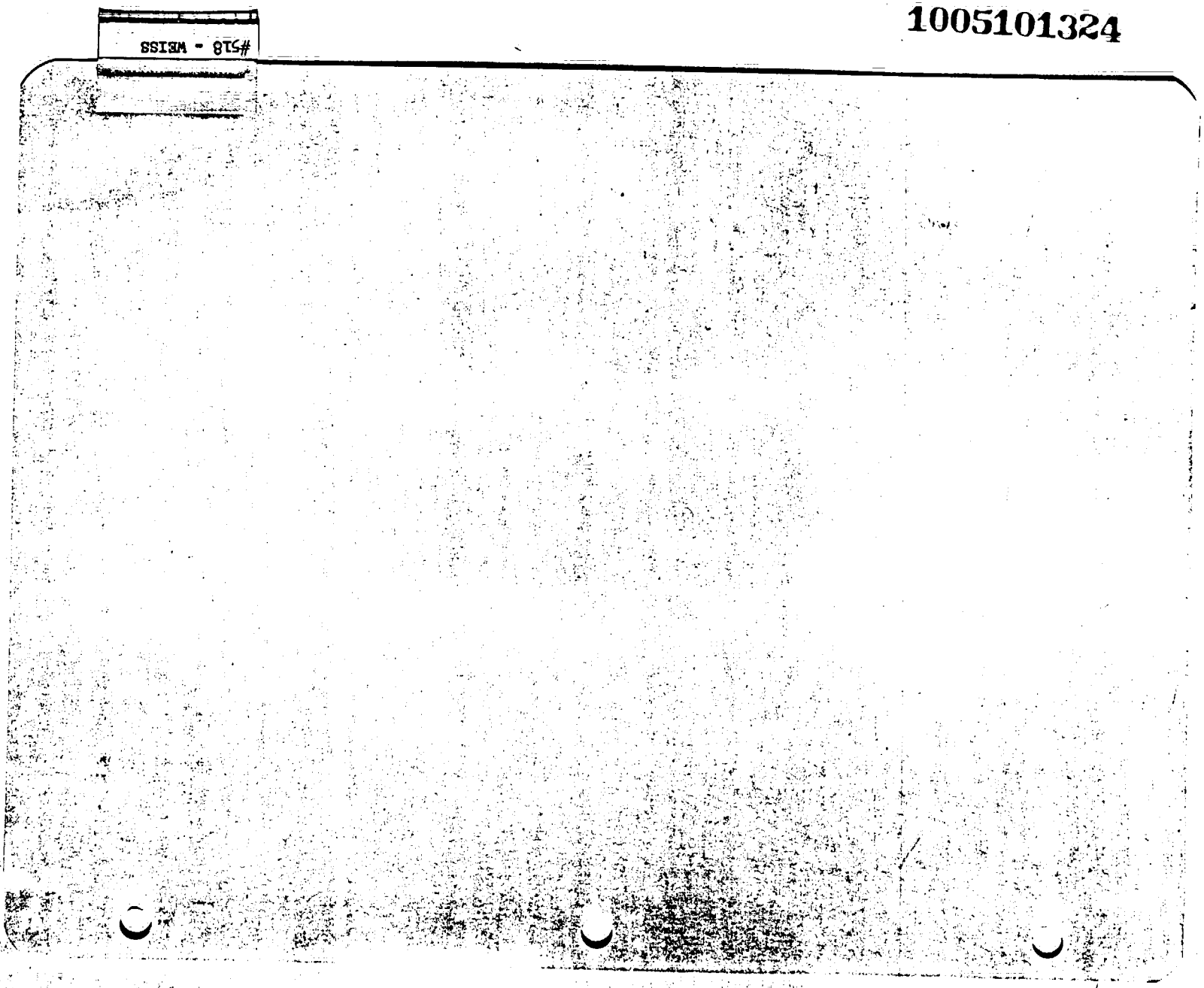

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THE COUNCIL FOR TOBACCO RESEARCH

CONFIDENTIAL

February 10, 1966

MEMORANDUM

TO: The committee comprising Dr. Andervont, Chm., Dr. Little and Dr. Cattell.

FROM: Robert C. Hockett

SUBJECT: New grant application from William Weiss, M.D. - No. 518

We enclose herewith a new grant application from Dr. William Weiss of the Philadelphia General Hospital, Philadelphia, Pennsylvania.

The bibliography indicates that the applicant has previously studied the effect of tobacco smoke solutions on Paramecium. The procedure for exposing paramecium to smoke is not described in the application. However, it seems doubtful that the effects of expired air would correspond to those in the tracheobronchial epithelium in normal smoking.

This proposal is being sent, at present, only to members of the committee but we plan to include it in the Agenda for the March meeting.

R. C. H.

Personal Opinion: "This proposal is related to general toxicity rather than to carcinogenicity. As such it does not seem to me to be very relevant to our problems. Certainly we should await the final report that has been promised before taking any action."

R. C. H.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

No. 518

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Andervont, Chm.
Dr. Little
Dr. Cattell

Application For Research Grant

Date: November 16, 1965

1. Name of Investigator: William Weiss, M.D.

2. Title: Clinical Director, Pulmonary Disease Service

3. Institution & Address: Philadelphia General Hospital
34th St. and Curie Ave.
Phila., Pa. 19104

4. Project or Subject: The bioassay of cigarette smoke toxins with Paramecium aurelia

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

The water-soluble toxins of cigarette smoke, mainly those in the gas phase, cause the death of Paramecium in sufficient concentration. The survival time is a measure of the concentration of toxins within certain limits. The response of Paramecium to smoke toxins is similar to the response of ciliated human tracheal epithelium as described by Ballenger. Therefore it may be possible to estimate the degree and duration of smoke toxicityfor tracheobronchial epithelium during and after smoking by determining the effect of expired air on Paramecium. The objective of this study is to try to develop a bioassay method.

Preliminary experiments show that Paramecium can be used in a hanging drop preparation in a Rose perfusion chamber to detect the presence of cigarette smoke gas phase in dilutions to at least 10 %. A standard dose-response curve will be developed to provide a means for estimating toxicity in expired air. Exploratory data suggest that the toxicity detectable by protoplasm rapidly disappears after smoking ends, using death of the organisms as the end-point. Photographic means will be used in an attempt to develop a motility endpoint.

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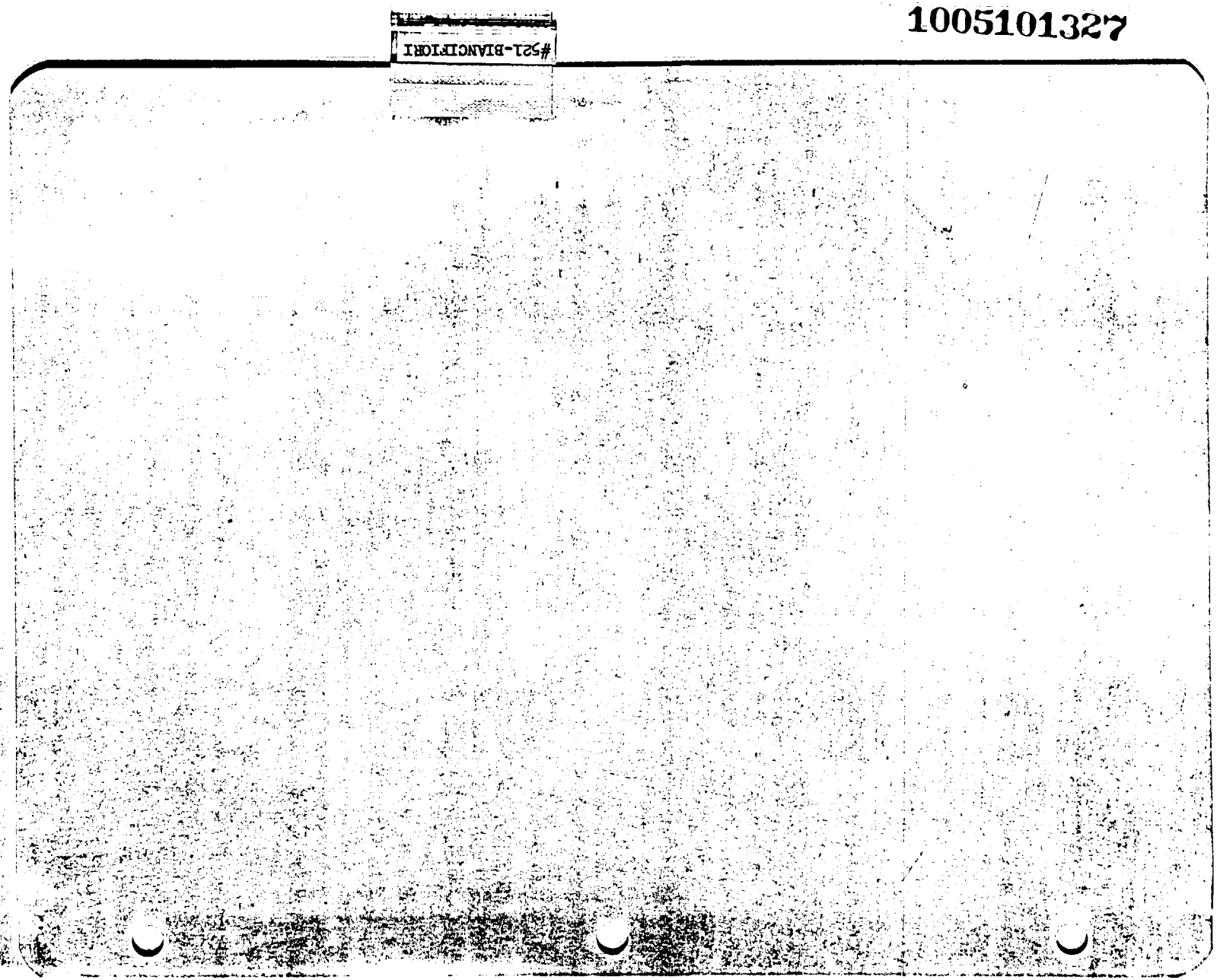

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HE COUNCIL FoR TOBACCO RESEARCH CONFIDENTIAL

ebruary 14, 1966

TO: The committee comprising Dr. Andervont, Chairman, Dr. Little and Dr. Jacobson.

ROM: Robert C. Hockett

UBJECT: New grant application from Cesare Biancifiori, M.D. - #521.

e enclose herewith a new application from Dr. esare Biancifiori of the University of Perugia, Italy. Ten eprints by Dr. Biancifiori are also enclosed.

visited this laboratory in June 1965 with Dr. old L. Stewart of the National Cancer Institute. Dr. Stewart xpressed a high opinion of the research group at the Perugia nstitute of Pathology, and has signified his willingness to te a comment on the proposed study for our SAB. This will be orwarded when received.

This application is being sent at present only to mbers of the committee, but we plan to include it in the agenda the March meeting.

C.H.

CH:ek

ncls.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

#521

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Andervont, Chm.
Dr. Little
Dr. Jacobson

Application For Research Grant

Date: January 17, 1966

1. Name of Investigator: CESARE BIANCIFIORI, M.D.

2. Title: Division of Cancer Research

3. Institution & Address: University of Perugia
P.O. Box 167
Perugia, Italy

4. Project or Subject: Lung tumorigenesis by isoniazid (INH), its metabolite hydrazine sulphate (h.s.) and derivatives of hydrazine.

New potential carcinogens for the human lung (INH and h.s.) have been demonstrated in previous experiments on mice. The aims of this research project are:

I. To study whether the carcinogenic action of INH and h.s. will take place in species of laboratory animals other than mice; and

II. To study whether other derivatives of hydrazine are carcinogenic for mice.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

I. INH, a widely-used drug in the treatment of tuberculosis, and hydrazine, its principal metabolite, have been shown to be carcinogenic in various substrains of mice (J. Juhasz et al.: Ztschr. Krebsforsch. 62, 186-196, 1957; L. Severi: Nature 192, 217-218, 1961: Cancer Bull. 16, 79, 1964; C. Biancifiori et al.: Nature 194, 488-489, 1962; Lav. Anat Pat. Perugia 23, 115-128, 1963; ibidem 23, 209-220, 1963; Brit. J. Cancer 18, 543-550, 1964).

Recent experiments have shown that h.s. is carcinogenic also for rats (C. Biancifiori et al.: in press), while C57BL/Cb/Se substrain mice have been shown to be resistant to the carcinogenic action of INH and of h.s. (unpublished data).

In order to study whether the carcinogenic action of INH and h.s. (its metabolite) will take place in species of laboratory animals other than mice, the following experiments will be carried out.

A. Carcinogenic action of INH and h.s. in rats.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

(5. IA continued)

Tests. 300 rats:
100 (50 m. and 50 f.) treated with INH and vitamin B6;
100 (50 m. and 50 f.) treated with
100 (50 m. and 50. f.) untreated as controls.

Treatment. INH; Each rat,, at 8 weeks of age, will, receive 1.0 ml of 2.0 per cent aqueous aolution daily by stomach tube, for 24 weeks. A sufficient amount of vitamin B6 will be added so ae: to prevent the polyneuritis frequently caused by this chemical and in this way avoid a high early-death rate among the animals due to this complaint.

h.s.: each rat, at 8 weeks of age, will receive 1.0 ml of 1.13 per cent aqueous solution daily by stomach tube, for 24 weeks.

Examination. All rats will be allowed to live for about 80 weeks, when they will be killed for examination. The lungs, liver, adrenala, ovaries and the organs suspected of tumours will be removed for histological examination. The lungs will be examined with he aid ofapecial technical procedures.

B. Carcinogenic action of INH and h.z. in golden hamsters.

Tests. 300 golden hamsters:
(50 m. and 50 f.) treated with INH and vitamin B6;
100 (50 m. and 50 f.) treated with h.s.;
100 (50 m. and 50 f.) untreated as controls.

Treatment. INH and h.s. as in IA. The dose will be varied in relation to the weight of the animals.

As in IA.

C. Carcinogenic action of INH and h.s. in rabbits.

t. 120 rabbits:
40. (20 m. and 20 f.) treated with INH and vitamin B6;
40. (20 m. and 20 f.) treated with h.s.;
0 (20 m. and 20 f.) untreated as controls.

tmeRt., INH and h,. s. as in IA. The dose will be varied in relation to the weight of the animals.

E ination. All rabbits will be allowed to live for about 104 weeks, when they will be killed for examination. The same organs as in IA. will be removed for histological examination.

D.B. Clayson et al. (International Conference on Lung Tumours in Animals, Perugia., 24th to 29th June, 1965) have shown, that some

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

(5. II continued)

derivatives of hydrazine are carcinogenic for BALB/c/Cb/Se substrain mice. Benzoyl hydrazide, 2-methoxybenzoyl hydrazide and 4-methoxybenzoyl hydrazide increased the incidence of lung tumours; iproniazid and phenylhydrazine hydrochloride were the least effective.

In order to study whether other derivatives of hydrazine are carcinogenic for mice, the following experiments will be carried out. In these experiments BALB/c/Cb/Se and C57/Cb/Se substrain mice, susceptible and resistant respectively to the induction of lung tumours by INH and h.s., will be used.

A. Carcinogenic action of P nitro benzoyl hydrazide.

Tests. 200 BALB/c/Cb/Se substrain mice:
100 (50 m. and 50 f.) treated:
100 (50 m. and 50 f. untreated as controls;

200 C57BL/Cb/Se substrain mice:
100 (50 m. and 50 f.) treated;
100 (50 m. and 50 f.) untreated as controls.

Treatment. The drug will be administered six times weekly in aqueous solution by stomach tube. The size of the daily dose will be determined from the results of preliminary toxicity tests. The treatment will be continued for 20 weeks.

Examination: All mice will be allowed to live for as long as possible and will be examined at their natural death. An untreated mouse of equivalent age will be killed whenever a treated mouse dies, in order to match the experimental and untreated groups as closely as possible. The lungs, liver, adrenals, ovaries and all the organs suspected of tumour will be removed for histological examination. The lung will be examined using special technical procedures.

B. Carcinogenic action of phenyl ethyl hydrazine sulphate.

Tests. 200 BALB/c/Cb/Se substrain mice:
100 (50 m. and 50 f.) treated;
100 (50 m. and 50 f.) untreated as controls;

200 C57BL/Cb/Se substrain mice:
100 (50 m. and 50 f.) treated;
100 (50 m. and 50 f.) untreated as controls.

Treatment and Examination. As in IIA.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan: (Per year)

a.	Salaries	$ 4,300.00
b.	Expendable Supplies	2,900.00
c.	Other Expenses	300.00
d.	Permanent Equipment *	2,500.00
e.	Overhead (15% of a, b, c)	1,100.00
	Total	11,100.00

(* 700 animal cages and 10 racks for cages: $2,500.00)

7. Anticipated Duration of Work: A period of three years will be necessary for the completion of the project - from March 1, 1966 to February 28, 1969.

8. Facilities and Staff Available: All the facilities existing at the Division of Cancer Research are available for this research project. The Division occupies two buildings, one of three floors and the other of four. The animal centre includes 32 rooms; mouse-rooms, storage-rooms, cold-rooms, fully-equipped food production unit and various services. In the animal centre (14) inbred substrains of mice, 3 of rats, 1 of golden hamsters and 1 of rabbits are maintained.

The Division also includes fully-equipped laboratories for histology (6 rooms), histochemistry and cytology (6 rooms), virology and tissue culture (13 rooms), biochemistry (9 rooms), library and offices for investigators. Of the staff employed in the Division, one histological technician and one animal caretaker will be available full

9. Additional Requirements: time for this project.

NIL

10. Additional Information (Including relation of work to other projects and other sources of support):

The demonstration that INH and h.s. induce pulmonary tumours in mice is of extreme theoretical importance insofar as human pathology is concerned, and it must be developed step by step through the study of the carcinogenic action of these two chemicals in laboratory animals other than mice. Experiments along these lines are an absolute requirement at the present time in view of the progressive increase in lung tumours in human beings and of the widespread therapeutic and prophylactic use made of INH in human tuberculosis.

Collateral studies with hydrazine derivatives have the aim of testing their carcinogenic action and of analyzing more closely the mechanism of action of INH and hydrazine as carcinogenic agents in living hosts.

Signature: Cesare Biancifiori

Dr. Cesare Biancifiori, M.D.
Director of Project

Prof. Lucio Severi, M.D.
Director, Division of Cancer Research,
Perugia, Italy.

Signature of Official Institution

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REDACTED MATERIAL

CURRICULUM VITAE

Dr. Cesare Biancifiori, Senior Assistant, Division of Cancer Research, University of Perugia. REDACTED Italy.

Present Citizenship: Italian. Sex: Male.

a. Education experience

Degree.	Institution. conferring	Fields	Year.
Baccalaur.	Liceo Classico "C. Tacito" Terni	---	1939
	University of Perugia	Medicine	1945
Specialist	University of Rome.	Resp. Dis.	1959
L.D.	Ministry of Public Instruction.	Morbid Anat.	1956

b. Other research training experience:

	Nature	Year
Institute of Morbid Anatomy and Division of Cancer Research, Perugia	Cancer rosearch in animals and in human beings	1953/1965
Chest Hoap. INPS Perugia	Resp. dis. and tubero.	1946/1965

Fields of present and/or scientific interest:

Carcinogenic action of INH and related compounds in laboratory animals. Cancer of the lung in human beings, respiratory diseases and tuberculosis.

Supplementary information:

Dr. Biancifiori has published 65 papers on various subjects. The most recent of them regarding the carcinogenic action of INH and its derivatives. He has visited several institutions for Cancer Research. Holland and in Great Britain.

Since 1959, Dr. Biancifiori has been acting vice-director of the Chest Hospital, Perugia. He has worked with Dr. G.M. Bonser in the Department of Experimental Pathology and Cancer Research, School of Medicine. Leeds, and continues to co-operate with her; and now, also, with Dr. D.B. Clayson of the same department in Leeds. In collaboration with Dr. Bonser he has published various papers on mammary cancer induced by chemical carcinogens in mice under different hormonal status.

Since 1959 Dr. Biancifiori has been studying the carcinogenic action of INH, hydrazine and its derivatives. Several papers have already been published on this problem and other will be in the near future. In some of these papers Dr. D.B. Clayson was co-author, and he is still collaborating.

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PAPERS FOR WHICH REPRINTS ARE NOT YET AVAILABLE

1. Histogenesis of pulmonary tumours by hydrazine sulphate in BALB/c/Cb/Se substrain mice. (In collaboration with F.E. Giornelli-Santilli, U. Milia and E. Bucciarelli)
   Presented at:
   International Conference on Lung Tumours in Animals. Division of Cancer Research, University of Perugia, 24th to 29th June, 1965.
   In press in the Proceedings of the Conference.

2. The induction of pulmonary tumours in BALB/c/Cb/Se mice by derivatives of hydrazine. (In collaboration with D.B. Clayson, U. Milia and F.E. Giornelli-Santilli)
   Presented at:
   International Conference on Lung Tumours in Animals. Division of Cancer Research, University of Perugia, 24th to 29th June, 1965.
   In press in the Proceedings of the Conference.

3. Pulmonary carcinogenesis by (oral and intraperitoneal) administration of hydrazine sulphate in newborn BALB/c/Cb/Se substrain mice. (In collaboration with U. Milia and F.E. Giornelli-Santilli)
   Presented at:
   6th Annual General Meeting of British Association for Cancer Research, Trinity College, Dublin, September 23-25, 1965.
   In press in "Lav. Anat. Pat. Perugia".

4. Pulmonary tumours in rats by oral hydrazine sulphate. (In collaboration with F.E. Giornelli-Santilli, U. Milia and L. Severi)
   Submitted for publication to "Nature".

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#522 - BICKS

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THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. INC. February 10, 19

MEMORANDUM

O: The committee comprising Dr. Jacobson, Chm., Dr. Lynch and Dr. Reiman.

FROM: Robert C. Hockett

UBJECT: New grant application from Richard 0. Bicks, M.D., and associates - No. 522.

e enclose herewith a new grant application from Dr. Richard 0. Bicks nd associates of the University of Tennessee College of Medicine, Memphis, Tennessee.

his proposal is being sent at present; only to members of the committee ut we plan to include it in the Agenda for the March meeting.

C. H.

ersonal Opinion. The effect of 2-4 D:nitrochlorobenzene on the respiratory system s only distantly related to our program and the price-tag appears very high

C. H.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

PHYSIOLOGY OF THE RESPIRATORY SYSTEM

No. 522

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Lynch
Dr. Reimann

Application For Research Grant

Date: December 23, 1965

1. Name of Investigator: Richard O. Bicks, M.D., FACP; E. William Rosenberg, M.D., FACP, Miguel A. Azar, M.D., Ph.D.

2. Title: Dr. Bicks: Assistant Professor, Department of Medicine
Dr. Rosenberg: Assistant Professor, Division of Dermatology
Dr. Azar: Research Fellow in Immunology

3. Institution & Address: University of Tennessee College of Medicine
62 South Dunlap
Memphis, Tennessee 38103

4. Project or Subject: Experimental Delayed Hypersensitivity Reactions of the Respiratory System.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

We have previously reported on the production of a chronic delayed hypersensitivity reaction in the gastrointestinal tract. With the use of a low molecular weight chemical 2-4 D:nitrochlorobenzene and an inert vehicle, Orabase, mucosal allergy of the contact type was localized in the colon and small intestine. The histological lesion in the colon showed mucosal ulceration with hemorrhage infiltration of the lamina propria with lymphocytes, perivascular cuffing, vascular engorgement and giant cell systems in the submucosa. This lesion is independent of the concentration of the contactant; depends on the frequency of application of the contactant and can be partially modified by immunosuppressive drugs. These lesions are immunologically specific and primary contact sensitization has been achieved, i.e., the lesion has been produced without prior skin sensitization. This work has been called the best experimental model of human ulcerative colitis to date. References to this work are cited below.

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We are currently investigating the specific nature of this immunological reaction, its localization on other parts of the gastrointestinal tract, and the relationship of gastric mucosal damage to parietal cell antibodies.

In the course of these studies, we made preliminary observations of this reaction localized in the respiratory tract. In four animals we produced a chronic pneumonitis and bronchitis. We propose to extend these observations in a systematic manner.

Pigs were chosen as the experimental animal because they react identically to man in terms of contact sensitivity. We plan to use 25 dewormed miniature pigs obtained from the Hormel Institute. They will be divided into five groups of animals. All animals will have a tracheostomy. Control group I will have this only. Control group II will be skin sensitized to a 2% solution of DNCB. Control group III will have Oradhesive powder blown into their tracheobronchial tree by IPPB. Experimental groups IV and V will be skin sensitized to DNCB and have plain DNCB and DNCB in Oradhesive instilled in their tracheobronchial trees. Each animal will be treated daily and sacrificed at intervals and examined morphologically. When a consistent histological lesion is produced the experiment will be repeated to establish a chronic lesion, and treatment response to antibiotics, immunosuppressive drugs and steroids studied. We hope to identify a chronic delayed hypersensitivity reaction in the respiratory tree.

Salaries are for the director, Dr. Bicks; two research associates, Dr. Rosenberg and Dr. Azar, one full time research technician and two part time animal caretakers. Expendable supplies include animal costs, care and feeding of animals and chemicals needed.

Dr. Bicks is a Diplomate, American Board of Internal Medicine and its Sub-Specialty Board on Gastroenterology. He is a member of the American Gastroenterological Association and a Fellow of the American College of Physicians.

Dr. Rosenberg is a Diplomate of the American Board of Dermatology and a Fellow of the American College of Physicians. He is a consultant to the U. S. Naval and Veterans Administration Hospitals.

Dr. Azar is an M.D. with graduate training in Pediatrics and a Ph.D. in Immunology.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0008

ertinent references include:

Rosenberg, E. W., and Fischer, R. W., Improved Method for Intraoral Patch Testing, Arch. Derm. 87:115, 1963.

2. Rosenberg, E. W., and Fischer, R. W., DNCB Allergy in the Guinea Pig Colon, Arch. Derm. 89:99, 1964.

3. Bicks, R. O., and Rosenberg, E. W., A Chronic Delayed Hypersensitivity Reaction in the Guinea Pig Colon, Gastroenterology 46:543, 1964.

4. Bicks, R. O., Rosenberg, E. W., et al, Further Observations on a Delayed Hypersensitivity Reaction in the Guinea Pig Colon, Gastroenterology, 48:425, 1965.

5. Bicks, R. Rosenberg, E. W., et al, Primary Induction of a Contact Hypersensitivity Reaction in the Swine Colon Gastroenterology, (In Press).

9. Bicks, R. O., Rosenberg, E. W. and Azar, M., A Contact Hypersensitivity Reaction in the Small Bowel of Swine

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	$ 60,000.00
b.	Expendable Supplies	5,000.00
c.	Other Expenses	3,000.00
d.	Permanent Equipment	3,000.00
e.	Overhead (15% of a, b, c)	10,200.00
	Total	81,200.00

7. Anticipated Duration of Work: 2 years @40,600 each

8. Facilities and Staff Available: All facilities of the Medical-Surgical Research Laboratories of the Kennedy Veterans Hospital, Memphis, Tennessee, and the University of Tennessee College of Medicine, Memphis, Tennessee, are available to us.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of support):

The work on the gastrointestinal tract is supported by Grant #09384 of the NIH, General Medicine Section of the National Institute of Arthritis and Metabolic Diseases. This will expire in December, 1968. We have established techniques, quarters, and skilled animal handlers. This supplemental grant will enable us to extend our observations to another system using procedures we have developed.

100510134O

Signature Richard Q. Bicka, M.D.

Director of Project

Homer F. Marsh

Business Officer of the Institution

Homer F. Marsh, Ph.D. Vice President

Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco/item/0000

25-0691
27-3311

RICHARD O. BICKS, M.D. F.A.C.P.
GASTROENTEROLOGY AND INTERNAL MEDICINE
THE TIFFANY BUILDING
100 WASHINGTON AVENUE
EMPHIS, TENNESSEE 38105

ebruary 12, 1966

r. W. T. Hoyt
Executive Director
ouncil for Tobacco Research U.S.A.
33 Third Avenue
ew York, New York 10017

r Mr. Hoyt:

n response to your letter of February 7, 1966, I am writing his letter to give you a budget breakdown for the two separate years involved.

nder category A, salaries: This would be $30,000.00, which ould represent salaries for three part-time investigators nd three full-time technicians.

nder B, expendable supplies: This would be $2,500.00, which ould be the cost of miniature swine, obtained from the Hormel nstitute of the University of Minnesota, which are weaned, ewormed, immunized against swine cholera and erysipelas. The ther expenses include their care and feeding at the Medical nd Research Laboratories at Kennedy Veterans Hospital.

nder permanent equipment would be cryostat and things for cost f triated thymidiine, which would be $1,500.00. Overhead would e $5,100.00 for a total of $40,600.00 per annum.

e have completed preliminary observation in four animals using acrifice at the end of 2¼ hours at the prior skin sensitization e have produced a lesion in the tracheal-bronchial tree characerized by lymphocyte and mononuclear cell infiltration peri ascular cuffing and destruction of the mucosa. These animals ere sacrificed at 24 hours and the problems of trauma were nticipated, but not handled properly. The year's budget would e to work out careful controls and to definitely show that a elayed hypersensitivity reaction of the respiratory tract ould be produced and to characterize its immunologic specicity.

hank you very much for your anticipated courtesy extended in ur behalf. If you desire further information, please feel ree to co tact me at any time.

ours truly,

c arih d O. Bicks, M D.

OB: p

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#523 - JONES

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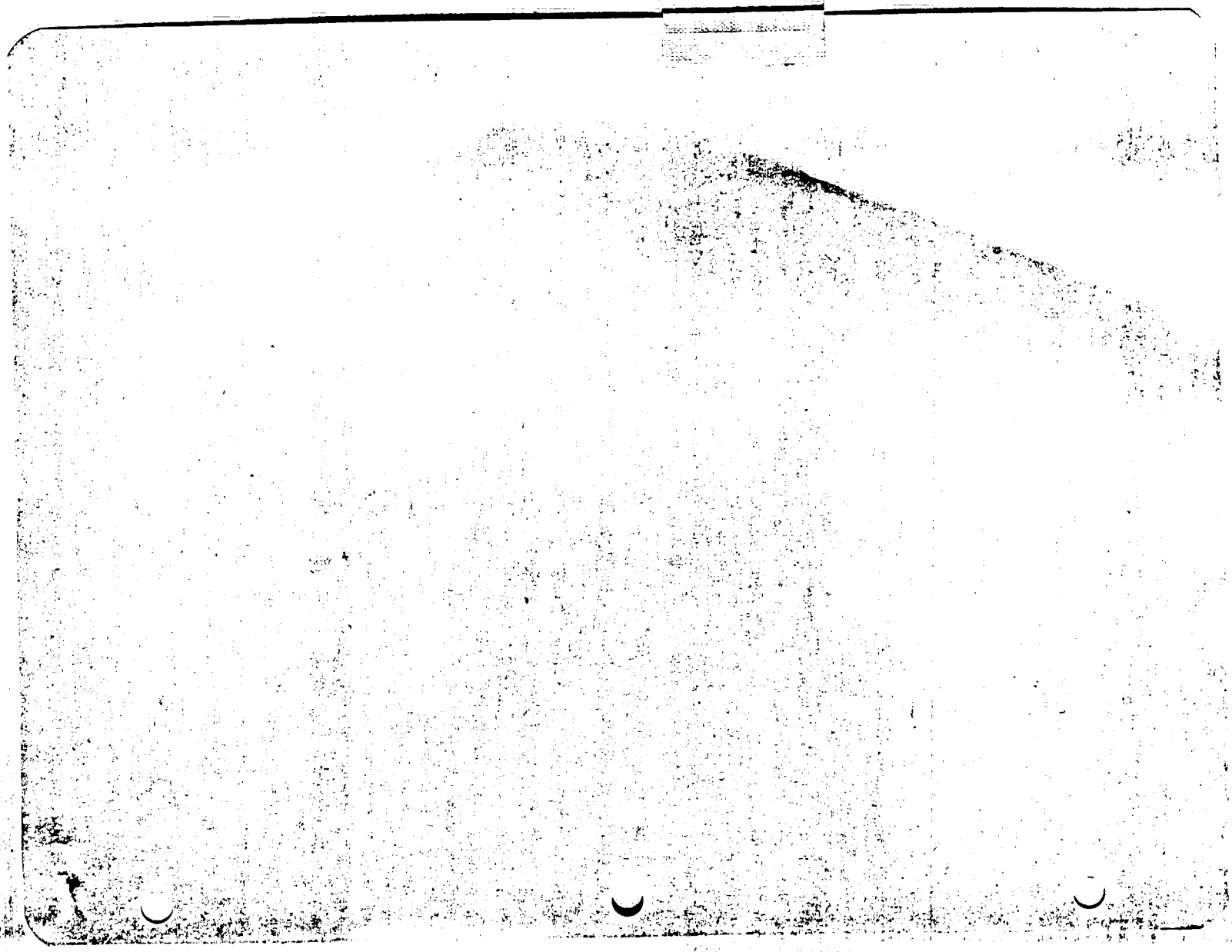

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CONFIDENTIAL

THE COUNCIL FOR TOBACCO RESEARCH - U. S. A.

February 16, 1966

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chm., Dr. Bing and Dr. Lynch.

FROM: Robert C. Hockett

SUBJECT: New grant application from Oswald R. Jones, M.D. - No. 523.

We enclose herewith a new grant application from Dr. Oswald R. Jones of St. Luke's Hospital, New York City.

This proposal was received following a number of conversations with Dr. Jones. Without the full background of these discussions, the application as it stands appears rather meager and, considered de novo, may not succeed in conveying an adequate picture of Dr. Jones' long clinical experience and related animal experimentation.

The theme and thesis of the proposal seem quite relevant to the basic approach of the Council to problems of chronic lung diseases such as emphysema. The Council has stressed the problem of predisposing factors such as (1) heredity and (2) the cumulative or repetitive effects of infections, that might ultimately explain why some smokers develop such diseases while others do not. Dr. Jones' special interest is in the predisposing effects of virus infections. He feels that long-range prospective studies, beginning with children, will provide the only means of solving these questions. Sooner or later such studies must be undertaken by somebody, he says, and continued for an extended period. Though this proposal speaks of four years, it is hardly to be expected that this will be time enough and another four will undoubtedly be needed , and perhaps yet another four. At the same time, some more immediate returns can be expected if even very young survivors of relatively severe pulmonary virus infections show evidence of persistent or even permanent impairment of pulmonary function and/or if occasional opportunities for post-mortem examination advance our knowledge of pathological changes in the lung attributable to particular infectious agents.

Jones asserts that practically no investigation of this type has ever been done before and I have found no basis for disputing him.

An undertaking of this kind would be an ambitious one for the Council because of its nature. It would nevertheless provide a pioneering and leadership opportunity. Certainly it should be considered without any illusions.

Aside from consideration of the plan and concept, the Board will have to evaluate the capabilities of the investigators and the suitability of the institution for an investigation of such magnitude and character. It may not be possible to do this adequately in one sitting. Dr. Jones has offered to appear before the whole Board for further discussion or to meet with any committee thereof which may be designated. I feel personally that this would be a wise step.

Since Dr. Jones is no longer a young man, it seems important that he should have collaborators both interested in and capable of carrying the study through if his leadership should be lost.

R. C. H.

10051343

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

DR. OSWALD R. JONES
71 EAST SEVENTY-FIRST STREET
NEW YORK 21, N.Y.

February 3, 1966

Dr. Robert Hockett
Council for Tobacco Research
633 3rd Avenue
New York City

Dear Doctor Hockett:

Thank you for your letter notifying me that you had received my request for a research grant. I was wondering whether the members of the council would get a clear idea of what we were after and how we were going to obtain our results.

As you know from our past talks, our objective in this project with children is the same objective which we are following in our dog work at Ithaca, namely to prove that the virus plays a large part in destroying the physiology of lungs. These changes, which we believe occur in childhood, may well lead to the ultimate development of pulmonary emphysema. In children we believe that the viruses of measles (morbelli), influenza, respiratory syncytial, chicken pox and adenovirus play a basic part in destroying the parenchyma of the lungs. The changes following these virus invasions may well take many different forms. Certain viruses (syncytial) seem to affect the bronchioles, others, measles and chicken pox oft times help destroy lung parenchyma.

Studying children from early life gives us many advantages such as a reliable history of childhood infections and proof from blood sera as to the type of virus invasions which has occurred.

By reason of sputum cultures, antibody titres, and if the tissue is available virus cultures, we hope to prove the hypothesis that many types of lung disorders can be traced to early viral infections most important pulmonary emphysema.

I am looking forward to the decision of the council on our request for a research grant.

Kindest regards.

Sincerely,

Oswald R. Jones

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VIRUS, Previous Infections

HE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

0. 523

UCCESSOR TO THE

TOBACCO INDUSTRY RESEARCH COMMITTEE

633 THIRD AVENUE

NEW YORK, N. Y. 10017

COMMITTEE:
r. Jacobson, Chm,
r. Bing
r. Lynch

A.pplication For Research Grant

ate: January 11, 1966

t Name of Invstigator: Oswald R. Jones, M.D.

2 Titl.: Director of Researoh Project

3. Institution & dress: St. Luke's Hospital - West 113th Street, New York City

4. Project or 5ubject:

Carbon monoxide uptake and diffusion capacity in children. This test together with others will be used to evaluate pulmonary function in children starting at the earliest age of cooperation. This evaluation of pulmonery function will be applied to normal children, those with asthma, bronchitis, or chronic pneumonia, which conditions were subsequent to infections of all kinds, particularlythose caused by known viral agents.

5 Detailed Plan of Procedure (Us. additional Rages if mor space is required)

Measurements of carbon, monoxide (CO) uptake and diffusion capacity(1): - have been modified to obviate painful arterial puncture by the use of nd tidal PCO at rest (2,3,4,5) and during. graded' hyperventilation(6)'. Application of these methods in the dog, and in normal humans and patients with chronic chest diseases. have established the relationship between CO uptake and diffusion as graded hyperventilation in normal and diseased, adults (7,10 supporting 8,9).

Normal children will be grouped according to age and size and normal values for CO uptake and diffusion established as controls. Children with asthma, bronchitis and post viral pneumonitis: Will be studied and the results compared

Th objectives of the study are: (1) to establish normal values for CO uptake and diffusion capacity at rest and during hyperventilation in children. (2) To study the relationship of asthma, bronchitis and viral pneumonitis on diffusion capacity in children as a background for the development of chronic pulmonary insufficiency.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

(a) 12,000 full time physiologist
3,600 part time pediatric cardiologist
4,800 technician

(b) 2,000 medical gasses, valves, tubing, bags, etc.

(d) 5,700. CO analyzer 2,800
Recorder 2,900 5,700.

a.	Salaries	20,400.
b.	Expendable Supplies	2,000.
c.	Other Expenses	3,600.
d.	Permanent Equipment	5,700.
e.	Overhead (15% of a, b, c)	3,900.
	Total	35,600

7. Anticipated Duration of Work:

This study is expected to require four years for completion of the above outlined objectives

8. Facilities and Staff Available:

The cardiopulmonary laboratory of St. Luke's Hospital is a recognized research laboratory in pulmonary diseases with four full time physicians, three blood gas technicians and all equipment and facilities to perform the studies outlined. New CO measurement and recording equipment is requested for specific application to small children and infants.

The pediatric department of St. Luke's Hospital is headed by a full time director and operates an active inpatient service of fifty-four beds with 1500 patients per one year, as well as an outpatient department with more than 30,000 visits per one year. A full time pediatric cardiologist will devote part time (1/3) to this project.

9. Additional Requirements:

Antibody titre for viruses. Fluorescent antibody search in tissues obtained by biopsy or at post mortem, also cultures of the above tissues for presence of bacteria and viruses.

10. Additional Information (including relation of work to other projects and other sources of support):

As mentioned CO uptake and diffusion capacity techniques have been developed in this laboratory which have been validated in adults and which are specifically applicable in children as described.

Signature [signature]
Director of Project

[signature]
Business Officer of the Institution

Executive Director

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7. Krstulovic, V.J., Felton, C.P., Bell, A.L.L.: Dynamic diffusion study in group of healthy humans. Effect of respiratory rate. - In preparation

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R: REDACTED MATERIAL

CURRICULUM VITAE

LUCY H. SWIFT M.D.

REDACTED

: New York City, Barnard College, New York City 1949, A.B., College of Physicians & Surgeons, Columbia University, N.Y.C. M.D. 1953. Mary Imogene Bassett Hospital, Cooperstown, N.Y. 1953 (July-Dec.) St. Luke's Hosp. N.Y.C., 1960-62, clin. fellow pediatric cardiology 1962-1964, Presbyterian Hosp. Babies Hosp. N.Y.C., Res. Assoc. pharm. 1954-1955 Div. Exp. Chemotherapy, Sloan-Kettering Inst. N.Y., Med. Lic. N.Y.S. 1962, Am. Bd. of Ped. Dipl. 1965, Instr. Pediatrics Columbia Univ. 1964-, Asst. Att. Ped.(Cardio) St. Luke's Hosp. 1964-, Ass. Ped. Columbia-Pres. Med. Center, N.Y.C. 1964 - Assoc. Visiting Ped., Harlem Hosp. N.Y. 1965-, Med. Soc. of County of N.Y., N.Y.State Med. Soc. Amer. Med. Assoc.

References

1. Swift, L.H., and Griffiths, S.P.: Cardiac Arrhythmias in the First Year of Life; The Heart Bulletin 14: 113-116, 1965 (Nov. Dec.)

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REDACTED MATERIAL

CURRICULUM VITAE

OSWALD R. JONES, M.D.

REDACTED

Waterbury, Conn. B.S., 1919 Yale College, M.D. Columbia 1923. St. Luke's Hospital., N.Y. med. 1923 25, Bellevue Hosp.,, N.T. chest serv. 1925-26, ass't path. St. Luke's Hosp., 1926-27. Inst. in Med. Col. Univ. 1927 32. Assoc. in Med. 1932 37.. Asst. Clin. Prof.. of Med. 1937r62. - F.A.C.P. Dipl. Lat. Med., Ac. Med.,, Dir. Cardio Respiratory Lab. St. Luke's. Cons. phy. St. Luke's., Cons.. Phy. chest dis. Southaapton Hosp.., Cons. phy. chest service Bellevue Hosp.

References:

. Jones,. O.R.: Measles - A Cause of. Emphysema., An Hypothesis oncerning Chronic Pulmonary Emphysema, a Possible Cause and its Preventi,on, Am. Rev. Resp. Dis. Vol. 87, 4, Apr.. 1963

, Jones, O.R., Platt, W.D., and Ataill, L.A. – Miliary Tuberculosis Caused by Intravenous Self-Injection of Tubercle Bacilli, Treated Successfully with Streptomycin Therapy., Am. Rev. of T.B., Vol.. 60, No.. 4 Oct. 1949

Jones, O.R. and Courn and A. - The Shrunken Pulmonary Lobe with Chronic Bronchiectasis., Am. Rav. of T.B. Vol.. 28,. No.. 3 Sept. 1933

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ones, O.R. and Burford, G.E. - Massive Atelectasis following Cyclopropane Anesthesia - Jour. A.M.A. Vol. 110, Apr. 2, 1938..

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R: REDACTED MATERIAL

CURRICULUM VITAE

VELJKO JOSEPH KRSTULOVIC, M.D.

REDACTED

Med. School of Univ. of Zagreb, Yugoslavia 1941. Int. Ped. Derm. & Gyn Univ. Zagreb 1941-42 Chief Army Hosp. Zagreb. 1942-43. Res. Int. Med. Univ. Hosp. 1951-54 Res. Cardiology Univ. Hosp. 1955. Bd. of Int. Med. 1957. 1951-57 Inst. in Med. at Medical School, Univ. of Zagreb. 1957-60 Chief Cardiopulmonary Section of Int. Med. Polyclinic at Medical School in Zagreb. 1957-60 Supervisor Pracitcal Course of Semiology at Med. School in Zagreb. Res. Fellow Cardiovascular Lab Cornell Medical School 1960-61 Research fellow in Cardiopulmonary Lab. St. Luke's Hosp. 1961-64. Res. Assoc. in Cardiopul. Lab. St. Luke's Hosp. 1965. Dir. Research Cardiopulmonary Lab. St. Luke's Hosp. 1966.

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QUIGLEY #524 - 10051101351

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THE COUNCIL FOR TOBACCO RESEARCH-U

ebruary 10, 6

MEMORANDUM

O: The committee comprising Dr. Lynch, Chm., Dr. Reiman and Dr. Little.

ROM: Robert C. Hockett

UBJECT: New grant applications from Lawrence F. Quigley, -Jr., D.M.D. and Dr. M. Cobb, III, Ph.D. - Nos. 524 - 525.

e enclose herewith two grant applications from Drs. Lawrence F. uigley and C. M. Cobb, of the Forsyth Dental Center, Boston, Massachusetts.

copy of a memorandum mailed to members of the committee last eptember is attached. This describes the action of the Board on Dr. Quigley's arlier application and the subsequent correspondence. It was accompanied by nclosures which we assume are still available in the files of the committee members.

r. Quigley has now come up with two relatively modest proposals, one oncerned with the correlation of "reverse" smoking with lung cancer and the other ith oral temperatures during cigar smoking, both normal and "reverse".

hese proposals are being sent, at present, only to members of the ommittee but we plan to include them in the Agenda for the March meeting.

C. H.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

CONFIDENTIAL

September 24, 1965

MEMORANDUM

TO: The subcommittee that originally considered the application of Lawrence F. Quigley, Jr., D.M.D. ($406), Drs. Lynch, Reimann, Kotin and Little.

FROM: Robert C. Hockett

SUBJECT: New informal inquiry concerning possibility of a modest grant to support a subsidiary part of their study of reversed smoking in the Caribbean.

You will probably all remember this proposal to study the phenomenon of "reverse smoking" including its pathological effects. The original budget estimate was $11,725 and the Board was sufficiently intrigued with the matter at its December 7, 1963 meeting to authorize a site visit by Drs. Wilson, Reimann and Hockett, which was made on February 21, 1964. This show of possible interest may have been responsible for the fact Quigley and his colleagues promptly submitted a revised budget including fees for most of the personnel involved, to a total of $34,170. The proposal was considered on March 7, 1964 and denied.

Our letter of notification (copy enclosed) indicated that expenditures could not be made at this level for the project, but left the door somewhat open for any new and perhaps more modest proposal in the future.

A new inquiry from Dr. Quigley was received in March 1965. A copy of this is enclosed with my reply of September 7th, and Quigley's response of September 15, 1965.

The question before the house is whether we should encourage Quigley to submit an application for either or both of the relatively modest studies briefly outlined in his letter at $4,314 and $7,392 respectively, without salaries for any of the senior personnel.

I would appreciate your advice on his question whether the time he might invest in preparing a proposal would be justified by the chances of favorable action.

A copy of his latest paper is enclosed. This confirms Stephano's claim that normal cigarette smoking does not raise temperatures in the oral cavity.

Enclosures: R. C.H.

1. Dr. Reimann's site visit comments of February 28, 1964
2. Quigley's budget letter of March 6, 1964
3. Our denial letter of March 18, 1964
4. Quigley letter of March 23, 1965
5. Hockett Letter of September 7, 1965
6. Quigley letter of September 15, 1965
7. Reprint - Quigley, Cobb and Hunt. Arch. Oral Biol. 10, 35 (1965).

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...FAL CAVITY

No. 524

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. Denied-3/7/64

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
COMMITTEE: 633 THIRD AVENUE
Lynch, Chm. NEW YORK, N.Y. 10017
Dr. Reinmann
Dr. Little

Application For Research Grant

Date: November 19, 1965

1. Name of Investigator: Lawrence F. Quigley, Jr., B.S., A.B., D.M.D., M.S..
C. M. Cobb, III, S.B., Ph.D.

2. Title: Affiliate, Forsyth Dental Center
Affilitte, Forsyth Dental Center

Address: Forsyth Dental Center
140 Fenway, Boston, Mass.

Project or Subject: ial Field Trip to determine the correlation of cancer and reverse cigar smoking.

5. Detailed Plan of Proc.dun (Use additional pags if more space is required.)

Palatal cancer is a rare disease and the etiology is unknown. Possible protective mechanisms may be supplied by the oral flora, salivary constituents, or the hereditary cytological pattern of the human organism. Despite the normaly rarity of palatal cancer, a great increase in its frequency has been reported by many workers who have studied reverse smokers (Shirokov, Lepp and Wenger, Redi, and Racugno, etc.

In an effort to reveal pertinent facets of the tobacco cancer relationship study has been carried out by these workers on reverse cigarette smokers in the Netherlands Antildes. The typical reverse smoker in this location has smoked six to twenty cigarettes a day for over thirty years. The Oral condition reveals a thick, tough, leathery palate, with marked leukoplakia and scarred burned areas. Gross examinations of several hundred reverse cigarette smokers in the Netherlands Antilles revealed an absence of malignancy. A detailed examination of 21 of these patients likewise revealed normal cytological and morphological characteristics. No cellular dysplasia was evident as determined by cytologic and biopsy reports. Necropsy statistics in the Netherlands Antilles showed the same incidence of palatal cancer as in the U. S. where no reverse smoking has been reported.

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LAWRENCE F. QUIGLEY, JR., D. M. D.
PRACTICE LIMITED TO ORTHODONTICS
7 RANDELEY ROAD
WINCHESTER, MASSACHUSETTS 01890
TEL. 729-7730

Section 5 (continued)

Reports of our studies are enclosed for your consideration.

The data of the Netherlands Antilles do not support the studies of other workers who have examined reverse cigar smokers almost exclusively. One explanation is that only reverse cigar smoking is carcinogenic. There is also the possibility that ethnic differences may be the cause of the different frequencies of palatal cancer.

What is proposed is an initial study to compare the effects of reverse cigarette smoking and reverse cigar smoking.

Much palatal carcinoma has been reported for reverse cigar smokers in Panama by research workers, Shirkov, et al. Preliminary correspondence with workers in this area (Ramon) has indicated that cooperation would be obtained from the different hospitals in this region.

It is proposed to carry out survey work in Panama to identify the effects of reverse cigar smoking. An effort will be made to discover patients suffering from carcinoma. The condition of the oral cavity for both normal and carcinogenic reverse cigar smokers will be studied with emphasis on the microscopic cellular changes of the epithelium and underlying connective tissue. The study will, of course, include complete examinations. The clinical picture, will be reinforced with data on other aspects or oral pathology.

The study will be pupported by statistical investigation of hospital records in the Panama region in order to confirm the validity of the clinical picture.

The results of the statistical and clinical studies should give an initial picture of the frequency of palatal carcinoma among reverse cigar smokers and the sequence of cytologic changes which lend eventually to cellular dysplasia in the palatal region. There is a possibility that causative factors may be clearly isolated.

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6. Budget Plan:

a.	Salaries	1100
b.	Expendable Supplies	1200
c.	Permanent Equipment	850
d.	Overhead (15% of a, b, c)	1232
e.	Other	3010
	Total	7392

7. Anticipated Duration of Work:

March 1, 1966 to March 1967

8. Facilities and Staff Available:

Dr. Gerald Sklar, Head of Dept. of Oral Patholgy, Tufts University School of Dental Medicine wishes to participate in the microscopic analysis of the cytologic smears and biopsies; Facilities and Staff at Lago Hospital, Aruba, N.A.; Facilities and Staff at Gorgas Hospital, Canal Zone, Panama; Facilities and Staff of General Hospital, David, Panama

9. Additional Requirements:

None

10. Additional Information (including relation of work to other projects and other sources of supply):

The project director, Dr. Quigley, has visited Aruba and South America on a three month periodic basis since 1960. He made a field trip to Panama in 1964 to familiarize Himself with the legal requirements of the Panamanian government. At the same time contacts were made with the medical, dental, and public health officials (Dr. Hernan Ramon, president of the Panamanian Dental Society was most cooperative). Letters of introduction were given to every hospital in Panama by the Dept. of Public Health. Preliminary field trips revealed localities inhabited by hundreds of reverse cigar smokers.

Signature Laurence J. Quigley Jr.
Director of Project

John R. Heinz, Director
Business Officer of the Institution

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LAWRENCE F. QUIGLEY, JR., D. M. D.

PRACTICE LIMITED TO ORTHODONTICS

7 RANDELCY ROAD

ESTIMATED COST OF INITIAL FIELD TRIP TO DETERMINE THE CORRELATION OF CANCER AND REVERSE CIGAR SMOKING

Personnel:	
ence F. Quigley, Jr.	no salary
Statistician for one month	S00
terpreter/social worker $20 per day	600
Expendable Supplies:	
base material and impressions $10/;t; 30 pts.	300
Polaroid film 2/rolls/pt, $5/roll x 30	300
Chest X-rays $5/pt • 30 pts	150
ilm 16 mm. OO ft.	
ilm 35 mm 2/pt/roll $5/roll	
Misc. Lab. Supplies	300
Permanent Equipment:	
Polaroid Camera for intra oral photo	750
Rental of Cine Kodak Camera	100
verhead::	1232
Misc:	
trip to Panana 2 workers	700
Living Allowance in Panama (30 days) $200 per worker	400
Rental of car $5./day	100
Cytological smears	
Biopsies	750
Typist	200
	7392

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R: REDACTED MATERIAL

LAWRENCE FRANCIS QUIGLEY, JR.

REDACTED

1945	B.S., United States Naval Academy, Annapolis, Maryland
1947	A.B., Navy Pacific University, Honolulu, Hawaii
1951	D.M.D., Tufts University School of Dental Medicine, Boston, Massachusetts
1955	Certificate of training (Orthodontic), Tufts University Graduate School
1958	M.S., Tufts University School of Dental Medicine, Boston, Massachusetts

1950-51	Teaching Fellow in Anatomy, Tufts University School of Dental Medicine
1951-53	Field Research Investigator in Periodontology, Tufts University School of Dental Medicine
1951-	Dental Consultant, Webster Clinic for Plastic and Reconstructive Surgery, Boston, Massachusetts
1957-	Research Associate, Tufts Cleft Palate Institute, Boston, Massachusetts
1961-63	Cleft Palate Consultant, Yale University Medical School, New Haven, Connecticut
1962-64	Affiliate in Dentistry, Forsyth Dental Center, Boston, Massachusetts
1964-	Affiliate in Orthodontics, Forsyth Dental Center

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Publications:

1. Marshall-Day, C. D.; Stephens, R. G; Quigley, L. F.: Periodontal Disease: Prevalence and Incidents, Journal of Periodontology, 26:185-203, 1955.

2. Webster, R. C.; Quigley, L. F.: Methods of correction of velopharyngeal sphincter incompetency using palatal and posterior pharyngeal tissues: Proposed system of classification, Journal of Plastic and Reconstructive Surgery, 18:474-489, 1956.

3. Webster, R. C.; Coffey, R. J.; Russell, J. A.; Nichols, R. S.; Quigley, L. F.: Method of examination and diagnosis of velopharyngeal incompetency - surgical aspects, Cleft Palate Bulletin, 8:7-9, 1958.

4. Webster, R. C.; Coffey, R. J.; Kelleher, R. E.; Quigley, L. F.: The otorhinolaryngologic effects of velopharyngeal incompetency, Cleft Palate Bulletin, 8:12-14, 1958.

5. Webster, R. C.; Coffey, R. J.; Russell, J. A.; Quigley, L. F.: Methods of surgical correction of velopharyngeal sphincter incompetency using palatal and posterior pharyngeal tissues: Proposed system of classification, Surgical Forum, 7:3-4, 1958.

6. Webster, R. C.; Quigley, L. F.: Pharyngeal flap staphylorrhaphy and speech aid as means of avoiding maxillofacial growth abnormalities in patients with cleft palate, A preliminary report, American Journal of Surgery, 96:820-822, 1958.

7. Quigley, L. F.; Shiere, F. R.; The role of salivary depressants in dentistry, Oral Surgery, Oral Medicine, Oral Pathology, p. 419-425, 1961.

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9. Hein, J. W.; Quigley, L. F.; Quigley, G.: Repeatable serial photography, Dental Radiography and Photography, 36:15-18, 1963.

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12. Quigley, L. F.; Avery, E.: Diagnosis and treatment of tongue thrust as a result of reverse swallowing, Journal of American Speech and Hearing Association, (in press)

13. Quigley, L. F.; Cobb, C. M.; Medico-electronic instrumentation for evaluation of palato pharyngeal competence in the normal and cleft palate patient, Journal of Plastic and Reconstructive Surgery, (in press)

14. Quigley, L. F.; Cobb, C. M.: Measurements of oral and burning zone temperatures during conventional and reverse cigarette smoking, Archives or Oral Biology, (in press)

15. Quigley, L. F.; Cobb, C. M.; Webster, R. C.: A technique for obtaining intra- and extraoral photographs, The Cleft Palate Journal, (in press)

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R: REDACTED MATERIAL

CAROLUS MELVILLE COBB

REDACTED

Lynn, Massachusetts

1944	S.B. Massachusetts Institute of Technology, U.S.A. (Chemistry)
1951	Ph. D. Massachusetts Institute of Technology, U.S.A. (Physical Chemistry)
1944-46	Research Chemist, Tennessee Eastman Corporation, Manhattan Project, Oak Ridge, Tennessee
1948-50	Teaching Fellow, Massachusetts Institute of Technology
1951-55	Senior Chemist, Ionics, Incorporated, Cambridge, Massachusetts
1955-60	Principal Scientist, Allied Research Associates, Boston, Massachusetts
1960-	Chief Chemist, American Science and Engineering, Incorporated, Cambridge, Massachusetts

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Carolus M. obb Publications

obb, C. M., Ashley; L. E., Sin le Particle Scatterin Functions or Latex Spheres in Water, J. Optical Society of America, 48, 9(4), pril 1958.

2 Cobb, C. M., Zaff, S., Ashley, L. E., Ectuilibria at Hic h Tern- eratures for the Formation of the Molecular Species C_2, C_3, Cu_2, nd Si_2, Kinetics, Equilibria and Performance of High Temperature ystems, Washington: Butterworths,, 1958.

obb. C. M., Zaff, S., Ashley, L. E., Equilibria at Hi h Tem- eratures for the Formation of the Molecular Species C_2, C_3, Cu_2, Si_2 Proceedings of the First Conference - Kinetics, Equilibria, nd Performance of High Temperature Systems, Butterworths, London 960.

4. obb, C. M., et al, A Fea sibility Study for a Liquid Analogue omputer - Phase I. sponsored by Geophysics Research Directorate, ir Force Cambridge Research Center, Air Research and Develo pment ommand under contrract number AF 19(604)-1509 (Allied' Research ssociates, Inc.,, Boston, Massachusetts - 1955).

5. obb, C. M., et al, A Feasibility Study for a Liquid Analogue omputer - Final Report. ponsored by Geophysics Research Direc- orate, Air Force Cambridge Research Center, Air Research and evelopment Command, under contract number AF 19(604)-1509 Allied Research Associates, Inc., Boston, Massachusetts - 1956)

obb, C. M., et al, Passive Total Momentum Experiment in Shot Marshmallow- April 1962, ASE. Document Number 254, American cience and Engineering, Inc.., Cambridge, Mas.s. - sponsored by the efense Atomic Support Agency, Sandia Base, Albuquerque, New exico, under contract numb.er DA-49-146-XZ-116.

7. obb, C. M., et al, Pinhole Camera. Experiment in Shot Marshmallow (nre-test report) - April 1962, ASE Document number 255, American cience: and Engineering, Inc., Cambridge, Mass. - sponsored by he Defense Atomic Support Agency, Sandia Base, Albuquerque, New exico, under contract number DA-49-1.46-XZ-116..

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Carolus M. Cobb Publications

9. Cobb, C. M., et al, Pinhole Camera Experiment in Shot Marshmallow (U) - August 1962, ASE Document number 301 (Revised), American Science and Engineering, Inc., Cambridge, Mass. - sponsored by the Defense Atomic Support Agency, Sandia Base, Albuquerque, New Mexico, under contract number DA-49-146-XZ-116.

10. Cobb, C. M., et al, Pinhole Camera Experiment in Shot Marshmallow (U) - September 1962, ASE Document Number 302 (Revised) American Science and Engineering, Inc., Cambridge, Mass. - sponsored by the Defense Atomic Support Agency, Sandia Base, Albuquerque, New Mexico, under contract number DA-49-146-XZ-116.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

Carolus M. Cobb Publications

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

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ORAL CAVITY

No. 525
Cf. #406
Denied-3/7/64

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Lynch, Chm.
Dr. Reimann
Dr. Little

Application For Research Grant

Date: November 19, 1965

1. Name of Investigator:
Lawrence F. Quigley, Jr., B.S., A.B., D.M.D., M.S.
C. M. Cobb, III, S.B., Ph.D.

2. Title:
Affiliate, Forsyth Dental Center
Affiliate, Forsyth Dental Center

3. Institution & Address:
Forsyth Dental Center, 140 Fenway, Boston, Mass.

4. Project or Subject:
Measurement of Oral Temperatures During Conventional and Reverse Cigar Smoking

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Palatal cancer is a rare disease and the etiology is unknown. Possible protective mechanisms may be supplied by the oral flora, salivary constituents, or the hereditary cytological pattern of the human organism. Despite the normal rarity of palatal cancer, a great increase in its frequency has been reported by many workers who have studied reverse smokers (Shirokov, Lepp and Wenger, Redi, and Racugno, etc.)

In an effort to reveal pertinent facts of the tobacco cancer relationship a study has been carried out by these workers on reverse cigarette smokers of the Netherlands Antilles. The typical reverse smoker in this location has smoked six to twenty cigarettes a day for over thirty years. The oral condition reveals a thick, tough, leathery palate, with marked leukoplakia and scarred burned areas. Gross examinations of several hundred reverse cigarette smokers in the Netherlands Antilles revealed an absence of malignancy. A detailed examination of 21 of these patients likewise revealed normal cytological and morphological characteristics. No cellular dysplasia was evident as determined by cytologic and biopsy reports. Necropsy statistics in the Netherlands Atnilles showed the same indidente of palatal cancer as in the U. S. where no reverse smoking has been reported.

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AWRENCE F. QUIGLEY, JR., D. M. D.
PRACTICE LIMITED TO ORTHODONTICS
7 RANGELEY ROAD
WINCHESTER, MASSACHUSETTS 01890
TEL. 729-77..0

ection 5 continued:

eports of our studies are enclosed for your consideration.

he data of the Netherlands Atnilles do not support the studies of other orkers who have examined reverse cigar smokers almost exclusively. ne explanation is that onl reverse ci ar smoking is carcino enic. her is also the possibility that ethnic differences may be the cause f the different frequencies of palatal cancer.

hese investigators have already reported on the temperatures of reverse igarette smoking. They now propose to extend the study to include everse cigar smokers.

uth palatal carcinoma has been reported for reverse cigar smokers in Panama by research workers (Shirokov et al). The studies on the issue temperatures produced during reverse cigarette smoking indicated hat cellular damage was partly caused by radiation burns. Higher tissue emperatures with greater burn damage are anticipated for the everse cigar smokers. Experimental measurements are necessary to confirm this.

t is proposed to measure the oral and tissue temperatures of during everse cigar smoking. The experiments will be designed to isolate ffects caused by infrared radiation from the glowing cigar tip.

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

6. Budget Plan:

a.	Salaries	840
b.	Expendable Supplies	~~1700~~ 1655
c.	Other Expenses	1100
d.	Permanent Equipment	
e.	Overhead (~~15~~ 20% of a, b, c)	719
	Total	4314

7. Anticipated Duration of Work:

March 1966 to March 1967

8. Facilities and Staff Available:

Facilities of Forsyth Dental Center
Laboratory at Sanborn Company, Waltham, Mass.

9. Additional Requirements:

None

10. Additional Information (including relation of work to other projects and other sources of support):

Please refer to Section 5.

Also, "Measurement of Oral and Buring Zone Temperatures During Conventional and Reverse Cigarette Smoking" Quigley, Cobb, Hunt Arch. of Oral B.

See, J.A.D.A. article, "Reverse Smoking in Caribbean Peoples"

Signature Lawrence J. Quigley Jr
Director of Project

Director of Admin.
Business Officer of the Institution

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LAWRENCE F. QUIGLEY, JR., D. M. D.

PRACTICE LIMITED TO ORTHODONTICS

7 RANGELEY ROAD
WINCHESTER, MASSACHUSETTS 01890
TEL. 729-7730

ESTIMATED COST FOR THE MEASUREMENT OF ORAL TEMPERATURES DURING CONVENTIONAL AND REVERSE CIGAR SMOKING

Personnel:	
Lawrence F. Quigley, Jr.	no salary
C. M. Cobb	no salary
Graduate student	840
Expendable Supplies:	
Thermocouples (Baldwin, Lima, Hamilton)	1500
Micro Miniature Thermocouples, $25/ther; 10 pts at 6 thermo/pt	
Electrical Supplies	150
Cigars 25 at 20c. ea.	5
Permanent Equipment:	
Rental of 4 track 8 channel recorder	900
Rental of camera equipment	200
Overhead	719
	$4314

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#526 - LARSON

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THE COUNCIL FOR TOBACCO RESEARCH, U.S.A.

CONFIDENTIAL

February 15, 1966

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chm., Dr. Reimann and Dr. Little.

FROM: Robert C. Hockett

SUBJECT: New grant application from Roger K. Larson, M.D. - No. 526.

We enclose herewith a new grant application from Dr. Roger K. Larson of the Fresno County Hospital, Fresno, California.

Another grant to Dr. Larson with Dr. Joseph R. McClintic on quite a different subject (No. 506) was activated on January 1, 1966 for a study at Fresno State College.

The present application is supported by the enclosed reprint of a preliminary study: Roger K. Larson and Martin L. Barman. The Familial Occurrence of Chronic Obstructive Pulmonary Disease. Ann. of Internal Med. 63, 1001 (1965).

This application is being sent at present only to members of the committee but we plan to include it in the agenda for the March meeting.

R. C. H.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

PULMONARY PHYSIOLOGY and CHRONIC PULMONARY DISEASES

No. 526
Cf. #506
Activated: 1/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Reimann
Dr. Little

Application For Research Grant

Date: JANUARY 26, 1966

1. Name of Investigator: ROGER K. LARSON, M.D., F.A.C.P.

2. Title: Chief of Medicine

3. Institution & Address: FRESNO COUNTY HOSPITAL
FRESNO, CALIFORNIA

4. Project or Subject: TO TEST MORE THOROUGHLY THE HYPOTHESIS THAT THE SUSCEPTIBILITY TO DEVELOP OR NOT TO DEVELOP EMPHYSEMA IN SMOKERS IS GENETICALLY DETERMINED.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

GENETIC INVESTIGATION OF EMPHYSEMA

I. Introduction

In a preliminary study supported by the Fresno County Tuberculosis and Health Association and reported at the American Federation for Clinical Research Meeting in January, 1965, the present investigator demonstrated an increased incidence of chronic obstructive pulmonary disease (C.O.P.D.) - like states in relatives of patients with C.O.P.D. as compared to the incidence in relatives of patients with other chest conditions not usually considered to be hereditary. (See attached summary tables and graphs.) This incidence was determined by detailed family history interviews of patients and the numbers were quite small. Only a few authors have reported on the familial occurrence of emphysema. In fact, McKusick, Goodman and Danks in an article entitled "Genetic Aspects of Respiratory Disease" published in 1963 commented on the lack of study of genetic factors in emphysema and gave the following explanation. "It is true that the conspicuous age-dependence of the disorder imposes difficulties. By the time the proband comes to attention, the parents are likely to be dead, the siblings are widely scattered and offspring are not yet old enough to show evidence of disease."

Two families studied in detail as part of this preliminary project showed such a high incidence of mild to severe chronic obstructive pulmonary disease as to suggest a genetic factor. (See attached tables and graphs.)

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

These studies are sufficiently encouraging to warrant a large ull-scale objective study of the role of heredity in chronic bstructive pulmonary disease.

II. Research Plan

. Specific Aims and Methods

It is the aim of this study to objectively assess the ncidence of obstructive pulmonary phenomena in siblings and parents of patients seen at Fresno General Hospital with far advanced chronic obstructive pulmonary disease and compare this ndicence to that found in a control population made up of the pouses of these relatives.

All patients admitted to the clinics or wards of F. C. H. ith chronic obstructive pulmonary disease not known to be secondary to other diseases, such as far advanced pulmonary uberculosis, pneumoconiosis, childhood allergic asthma, tc.,: wil l be includ ed in the study. Diagnos is wil l be stablished by history, physical examination, x-ray and pulmonary function studies in each case. The names and ddresses of all siblings and parents residing in the icinity of Fresno will be obtained by interview. These relatives will then be contacted and asked to appear with their spouse at the hospital for investigation. This investigation will consist of the following:

1. Filling out a questionnaire regarding symptoms, past history, occupational hist and smoking history (see attached form) also history.

Performance of at least three rapidly recorded tired inspired and expired vital capacities on a Collins 13. 5 L spirometer with a recording speed of 16 mm/sec. rom thi's the F. V. C., the F. E. V. 1% and MMF will be calculated for each patient. In addition, the ratio of mid-inspiratory to mid-expiratory flow rates will be obtained.

etermination of functional residual volume by the losed circuit helium technique.

sweat c1 o
the

6 h st x-ray.

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All those having significantly abnormal findings on the above screening procedures will be asked to return for more detailed studies to include: 1) arterial PO_2, PCO_2 and pH; 2) carbon monoxide diffusing capacity; and 3) bilateral tomograms of the chest.

The incidence of abnormal findings in the siblings and parents will be compared with the incidence of abnormal findings in their spouses as a control group. Conventional tests of statistical significance will be applied to any difference found. The mean values for each test result will also be calculated for each group and the difference in means tested by the Student t test. An additional analysis will be made after dividing the groups into smoker and nonsmoker. categories to eliminate any bias that might be introduced by this potent environmental factor.

B. Significance of this Research

The etiology of emphysema remains obscure. A large amount of money is presently being invested in investigation of the cause but almost no attention has been directed to possible genetic aspects. The present investigator has done previous work supporting the role of environmental factors (principally cigarette smoke) in the pathogenesis of emphysema. That this is not the only factor, however, is indicated by two facts: 1) a small number of patients with emphysema have never smoked and 2) most people who smoke do not get emphysema. It is the purpose of this study to explore the hypothesis that a genetic factor determines the susceptibility of an individual to environmental factors (particularly cigarette smoke) in the development of emphysema. If this should be substantiated, it might help to identify the population at greatest risk and aid physicians in counseling their patients in the prophylaxis of this increasingly common and devastating disease.

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Source: https://www.industrydocuments.ucsf.edu/docs/nkwp0000

6. Budget Plan:

a.	Salaries	14,840.00
b.	Expendable Supplies	1,000.00
c.	Other Expenses	
d.	Permanent Equipment	
e.	Overhead (15% of a, b, c) 10%	1,584.00
	Total	17,424.00

(See attached breakdown.)

7. Anticipated Duration of Work: Two years.

8. Facilities and Staff Available:

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of support):

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Signature Roger K Larson MD
Director of Project

Business Officer of the Institution H.L. Kaufmann, Administrator
Fresno County General Hospital

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ROPOSED BUDGET PER ANNUM FOR TWO YEAR PROJECT

PERSONNEL		
ulmonary fellowship ($\frac{1}{2}$ support of 4th year		
edical resident for work on project)		4,040.00
Technician full-time		, 00.00
enior Clerk-Typist		4 8 0.00
UPPLIES		000.00
	total	5
	+ 10% verhead	84.00
		[illegible]

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ADDENDUM

The criteria for diagnosis of chronic obstructive pulmonary disease, (C.O.P.D.) in the index cases and relatives will be a) a history of exertional dyspnea for at least one year, b) physical findings of obstructive airway disease such as expiratory wheezing on forced expiration, and c) pulmonary function abnormalities typical of obstruction -- in particular a F.E. V.1% below 70%, a midexpiratory flow rate (MMF) below 40% of the predicted value, a ratio of total lung capacity in liters to midexpiratory flow rate in liters/sec. of greater than 2 and a ratio of residual volume to total lung capacity of greater than 40%. Attempts will then be made to subclassify these cases into one of the following two categories:

a. Probable obstructive pulmonary emphysema (C.O.P.E.)

b. Probable chronic obstructive bronchitis (C.O.B.) without or with prominent bronchospasm ("asthmatic bronchitis")

Criteria for separation into these subgroups will be as follows:

A. Obstructive pulmonary emphysema -- all the criteria listed above for C.O.P.D. plus:

1. History: Relatively progressive or stable exertional dyspnea for at least one year.

2. Physical findings: Decreased breath sounds at both lung bases posteriorly, little expiratory wheezing.

3. X-ray: Attenuated vascular markings and/or prominent bullae on either conventional PA roentgenogram or tomograms.

4. Pulmonary function studies:

a. Evidence of expiratory collapse of bronchioles i.e. a ratio of midinspiratory flow rate to midexpiratory flow rate of > 2.5.

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b. Reduction in single breath carbon monoxide diffusion capacity < 60% of predicted normal.

B. Obstructive bronchitis -- all the criteria listed above for C.O.P.D. plus:

1. History: Prominent productive or nonproductive cough. Intermittent nature of exertional dyspnea.

2. Physical findings: Normal intensity of breath sounds at bases posteriorly. Prominent expiratory wheezing.

3. X-ray: Normal vascularity and absence or dearth of bullae.

4. Pulmonary function:

a. Ratio of midinspiratory flow rate to midexpiratory flow rate of < 2.5.

b. Relatively normal single breath carbon monoxide diffusion capacity -- > 60% of predicted normal.

c. Significant response to bronchodilator drug.

Patients will be scored according to the chart on the next page, and classified according to the highest score. In case the scores are within two points difference, the case will be classified as indeterminate.

Attempts will be made to secure autopsies on any index case or relatives who expire during this study. Lungs will be fixed in the inflated state with liquid 10% formalin at 25-30 cm. of pressure. Gross and microscopic sections will be taken from these specimens and examined with dissecting and standard microscopes respectively.

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Factors Favoring	(Chronic COPE Obstructive Pulmonary Emphysema)	Score	(Chronic COB Obstructive Bronchitis)	Score
History				
Dyspnea	Stable or Progressive	2	Intermittent	2
Cough	Mild	1	Prominent	1
Px				
Breath sounds	Diminished at bases	1	Normal	1
Wheezing	Absent	1	Prominent	1
X-ray				
Vascularity	Attenuated	1	Normal	1
Bullae	Prominent	1	Minimal	1
Function studies				
$\frac{\text{Midinspir. F.R.}}{\text{Midexp. F.R.}}$	> 2.5	2	< 2.5	2
$D_{LCO_{SB}}$	$<$ 60% of normal	1	$>$ 60% of normal	1
Response to Isuprel	Poor	1	Good	1

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TANDARD HISTORY QUESTIONNAIRE

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Sex

elative of:

elationship

Occupations

Childhood asthma

neumonia

lcohol consumption

moking history

. Age started.

. Types of smoking

. Average number of packs/day

. Still smoking.

. Age stopped.

Chronic cough

putum production

mop s

Chest pain

Dyspnea or Exertion

. uration

b. Stable

. Progressive.

. Intermittent.

. Amount of activity required to produce dyspnea

heeze

7. amily history (See additional form)

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8. Facilities and staff available

The complete facilities of the pulmonary function laboratory at Fresno General Hospital will be used for this project. This laboratory is equipped with the following items:

2 13.5 L Collins spirometers with high speed kymographs

2 Collins Residual Volume Carts with helium analyzers - one with balloon in a box system for single breath CO diffusion capacity test

1 Beckman GC 2A gas chromatograph for N_2, O_2, CO_2, and CO analyses

1 Radiometer system for pCO_2, pO_2, and pH determination of arterial blood

1 Beckman Spinco system for pCO_2, pO_2, and pH determination of arterial blood

1 Scholander apparatus

1 Sanborn 2 channel recorder, pressure transducer and Collins spirometer potentiometer for measurement of pulmonary compliance.

In addition, other hospital facilities such as clinic, x-ray, and pathology will be utilized.

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Name #	Age if Living	Age at Death	Emphysema	Asthma	Chronic Bronchitis	Lung Cancer	Chronic Cough	Chronic Dyspnea	Wheezing
Mother									
Father									
Brothers									
Sisters									
Sons									
Daughters									

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

February 8, 1966

CONFIDENTIAL

MEMORANDUM

TO: The committee comprising Dr. Reimann, Dr. Andervont and Dr. Little.

FROM: Vincent F. Lisanti

SUBJECT: Proposal from Dr. J. Manhold, D.M.D. - No. 529.

Dr. John Manhold of the New Jersey State Dental School has submitted a modest proposal which is estimated at eighteen months duration. His oral pathology service and register will provide source material for information needed by the health professions.

This proposal should be considered a pilot or feasibility study at two levels:

A. To relate the frequency of aberrant growths to smoking histories from material available and to extend this type of related study with biopsy material as it comes into his laboratory on a current basis.

B. To evaluate gingival biopsy tissues from a respircmetric point of view in both smokers and non-smokers.

This can become another component area of interest in our Oral Research Program. Outside consultative opinion is very favorably inclined both in need of this study and the qualifications of Dr. Manhold to do a serious thorough investigation.

Vincent F. Lisanti

P.S. Enclosed are four reprints.

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RAL PATHOLOGICAL DIAGNOSTIC

#529

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE

OMMITTEE:
Dr. Reimann, Chm.
Dr. Andervont
Little

630 THIRD AVENUE
NEW YORK, N.Y. 10017

Application For Resaarch Grant

Date: 31 January 1966

1. Name of Investigator: John H. Manhold, Jr., D.M.D., M.A., F.A.C.D., F.I.C.D..

2. Title: Professor and Director of Pathology and Oral Diagnosis for the College of Dentistry

3. Institution & Address: New Jersey College of Medicine and Dentistry
Jersey City, New Jersey 07304

Project or Subject: To study the purported relationship between smoking and changes in human oral tissue in vivo by routine microscopy, differential staining, and microrespirometer methods, and to further examine statistically two series of data presently in the principal investigator's possession.

Detailed Plan of Procedure (Use additional pages if more space is required.):

Introductory data -- The relationship between smoking and malignant or premalignant changes in bronchial and lung tissue has been much studied.. Additionally, oesophageal changes with smoking have been studied at some length by, among others Wynder and Brosland Auerbach[2] (who incidently also is one of the investigators who has published several articles on bronchial and lung changes associated with smoking).[3-7] The oral cavity per se has not been as well studied although many statistics and much conjecture exist in regard to a purported relationship between smoking and the occurrence of malignancy in this area. However, the statistics stem largely from linear correlations which continue to offer considerable controversy and the conjecture, along with some of the statistics, stem from animal experimentation done with the use of "smoke boxes' and/or the application of various carcinogenic agents. Actual human in vivo experimentation from other than the statistical viewpoint, is not extensive... Kirsch,[8] for example, has suggested the existance of a difference in salivary pH between smokers and non-smokers. Pindborg,[9] from the results of microscopic study of a small sample, has suggested that cigarette smoking produces a hyperparakeratotic condition in the oral cavity. Wrubel and Scopp,[10] using oral exfoliative cytologic methods have reported no change in the hard palate and buccal mucosa with Cessation of smoking. On the other hand, Zimmerman and Zimmerman[11], using the same method, have reported significant differences in oral tissue keratinization between smokers and non-smokers.

A certain lack of confidence exists among many pathologists in regard to oral exfoliative cytologic techniques. Peters[12] has demonstrated some basis for this lack of confidence, and Manhold et al[13] have demonstrated a further apparent unreliability of the oral cytologic technique by not being able to find histological evidence to corrobrate the cytologic results reported by Derbyshire and Uanhodi.[14]

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Nevertheless, these various studies by Kirsch, Pindborg, Wrubel and Scopp and Zimmerman and Zimmerman, when considered in relation to studies done by Cahn and associates,[15,16,17] suggest the possibility of more exact human in vivo investigation of the oral tissue-smoking problem. For example, Cahn, Eisenberg and Blake suggested in 1961 that it may be possible to distinguish potentially malignant epithelium from benign epithelium by study of the basement membrane with Periodic Acid Schiff[15] stain and in 1962 these same authors suggested that the existance of parakeratosis without glycogen may be "a sinister sign in the prognosis of white lesions of the mouth."[16] They also suggested in this same study that a difference in RNA could be seen with methyl-green pyronine.

It is proposed, therefore, to study the purported relationship between smoking and changes in human oral tissue in vivo by routine microscopy, differential staining, and microrespirometer methods, and to further examine statistically two series of data presently in the principal investigator's possession in the following manner:

PROCEDURE

Part I

It is proposed that a group of individuals who are heavy cigarette smokers and a group who are non-smokers have an area biopsied from the oral mucosa and that this biopsy be submitted to histologic study with H & E, Periodic Acid Schiff, Methyl-green Pyronine, and Feulgen Stain methods. Simultaneously part of these tissues will be subjected to tissue microrespirometry to ascertain whether any oxygen consumption difference appears to exist between the tissue of the smokers and non-smokers.

The staining techniques employed will be standard and will be utilized to compare with the results presented by Pindborg, Wrubel and Scopp, Zimmerman and Zimmerman and Cahn et al. The microrespirometer technique to be utilized here is fundamentally that initiated by Kirk.[18] It will employ the Stern-Kirk microrespirometer in the manner described by Manhold and associates,[19-26] and will allow comparisons with the values reported by these authors for normal and inflammed tissues.

Part II

In addition to this procedure the following is suggested:

Over the past seven years the proposed principal investigator has conducted a Biopsy Service for dentists of the State of New Jersey and environs. This service now processes in excess of 1,000 specimens per year. Over the past year and one-half there have been, among the biopsies submitted, 37 patients whom we have diagnosed as having lesions of a fundamentally hyperkeratotic nature; 8 patients who have been diagnosed as having invasive carcinoma; and there have been 2 patients who have been diagnosed as having leukoplakia. I propose first to contact the surgeons who submitted these specimens so as to gain pertinent data in regard to smoking habits, use of alcohol, and any medical or oral history which could be pertinent.

With these data for comparison, sections from blocks of these lesions will be cut and re-examined microscopically with appropriate H & E and differential staining as described above in Part 1. (If any significant trend appears from this preliminary perusal, additional information could later be gained by tracing all cases of this type back through the remaining six years of our Biopsy Service. This work could also then be expanded to study any epithelial hyperplastic change. Further work in this area would, as stated, depend upon the apparent fruitfulness of this preliminary survey and would however, constitute another area of endeavor, which would necessitate further planning and additional funds.)

Part III

Because production of oral carcinoma is thought by many to stem largely from irritation, and because changes in the normal physiology of components of the oral cavity can be thought to be part of this irritational factor and also because Kirsch[8]

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Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000

has suggested that a difference exists in the pH of the saliva of smokers and non-smokers, it might well prove interesting to ascertain whether this reported difference can be replicated in another sample. We have already accumulated data on a number of individuals and established a relationship between saliva, dental calculus formation and Blood Serum Phosphorus.[27] Thus we have pH and quantity of saliva on this group. We have, additionally but as yet unanalized, data on these individuals as to whether or not they are smokers. To examine these relationships will require merely a T-test or critical ratio on these data.

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R: REDACTED MATERIAL

JOHN H. MANHOLD, JR., B.A., D.M.D., M.A., F.A.C.D., F.I.C.D.

REDACTED

2. Professional History in Brief:

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3. Education:
B.A., University of Rochester 1940
D.M.D., Harvard University 1944
Fellow in Pathology, University of Rochester 1944
Certificate in Medical Aspects of Atomic, Bacteriological, Chemical Warfare: Army Chemical Corps School, Maryland 1950
M.A., in Psychology, Washington University 1956

4. Honors:
International College of Dentists (Fellow)
American College of Dentists (Fellow)
Society of the Sigma Xi (Member)
Omicron Kappa Upsilon (Member)

5. Biography in Who's Who in the East (since 1950), American Men of Science (Vol. II), and Leaders in American Science.

6.

b.

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c.

7. Positions (from present to earliest)
Professor and Director of Pathology and Oral Diagnosis, Seton Hall (now New Jersey) College of Medicine and Dentistry, 1957-; Secretary to the Executive Faculty, 1957-; Coordinator of Cancer Teaching, 1956-; Attending in Pathology, Jersey City Medical Center 1958-; Coordinator of Research 1956-1960.
Associate Professor and Director of Pathology, College of Dentistry, Seton Hall College of Medicine and Dentistry, 1957-1957.
Assistant Professor and Director of Pathology, College of Dentistry, Seton Hall College of Medicine and Dentistry, 1956-1957 (Interim Appointment).

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Assistant Professor and Acting Head of the Departments of General and Oral Pathology, Washington University School of Dentistry 1954-1956.

Research Officer and for several months Senior Research Officer Present, Aviation Psychology Laboratory and Research Pathologist, Naval School of Aviation Medicine, N.A.S., Pensacola, Florida 1952-1954.

Assistant Force Dental Officer, Fleet Marine Forces of the Pacific, 1951-1952.

Assistant Research Officer and Lecturer on Medical Aspects of Atomic Explosion, U.S.N.T.C., Great Lakes, III., 1950-1951.

Director, Cancer Teaching Program and Instructor, Oral Pathology, Tufts University College of Dentistry 1948-1950.

Private practice for a short time after World War II in which service was performed in the Navy for two years.

Designated by the Bureau of Medicine and Surgery of the United States Navy as "Qualified for Independent Research Duty".

Formerly Principle Investigator of U.S. Navy Research Project NM 001 057.11

8. Publications:

A. Books

1) INTRODUCTORY PSYCHOSOMATIC DENTISTRY by Manhold, J. H., Jr. Appleton-Century-Crofts, New York, 1956.
2) OUTLINE OF PATHOLOGY by Manhold, J. H., and Bolden, T. E. W. B. Saunders Co., Philadelphia, 1960.
3) Dental Clinics of North America, Contributing Author, W. B. Saunders Co., Philadelphia, November 1962, Chapter on "The Psychosomatic Process in Dental Disease".
4) Orban's Periodontia, Ed. 2, Contributing Author, C. V. Mosby Co., St. Louis, 1963.
5) Pharmacotherapeutics in Oral Disease, Contributing Author, McGraw-Hill Co., New York, 1964, Section on "Traumatic Lesions of the Mouth".
6) CLINICAL ORAL DIAGNOSIS by Manhold, J. H., Jr., McGraw-Hill, New York, 1965.

b. Papers (Abstracts are not included unless subsequently published as complete papers on the subject presenting substantially additional material.)

Early Research Reports:

1) Manhold, J. H., and Manhold, B. S.: A Preliminary Report on the Study of the Relationship of Psychosomatics to oral Conditions - Relationship of Personality to Dental Caries. Science 110:585, 1949.
2) Glickman, I., Turesky, S., and Manhold, J.: The Oxygen Consumption of Healing Gingiva. Jour. Dent. Research 29: 429, 1950.

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anhold, J. H., and Jones, M. B.: A Study of Social Attitudes n Relation to Dental aries Formation. Washington Univ. Dental Jour. XXII, #2, May 1956. (Recalled after acceptnce by Jour. Dent. Research because material covered in k INTRODUCTORY PSYCHOSOMATIC DENTISTRY which wasn in press. nd to be published before Jour. Dent. Research paper would. go to press.)

S. Navy Research Reports Published (1950-1955):

anhold, J.: A Preliminary Report on the Study of the Relationship of Psychosomatics to Oral Conditions II. Relationship of Personality to Periodontal Conditions. Great akes, XII., 1950.

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3) M 001 057.11.02 Dental Complaints of Flying Personnel, av. Sch. Av. Med., Pensacola, Florida, 25 November 1953.

Id) NM 001 057.11.03 Study of Psychosomatic Factors in Oral athology, Nav. Seh. Av. Med., Pensacola, Florida, 12 pril 1954.

5) M 001 057.11.04 The Relationship of Dental Caries to General ealth, Nav. Sch. Av, Med., Pensacola, Flcrida, 8 June 1954.

6) M 001 057.11.05 A Comparative Study of Several Methods of suring Dental Caries in Man. Nav. Sch. Av., Med., Pensacol, lorida, 2 September 1954.

apers since affiliating with Seton Hall:

17) anhold, J. H.: Biopsy from the Pathologist's Viewpoint. . Y. Jour. Dent. XXVII, #5, May 1957, pg. 193 ff.

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47) Manhold, J. H. and Manhold, B. S.: Final Report of An Eight-Year Study of the Efficacy of the Dental Aptitude Test in Predicting Four Year Performance in a New Dental School. Jour. Dent. Ed. 29:41, 1965.

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RELIMINARY REPORT OF RESPIRATION CHANGES IN RAT GLAND TISSUE A'S A RESULT. OF LIGATION. J.H. Manhold, Jr., T. B. Bolden, and M. L. Hall. The exorbital acrimal gland of the rat exhibits many similarities to the salivary gland, and is more advantageous for study. A normai respiratory quotient (Q_{O_2} = 0.41+ 0'. 14) was established for oxorbital lacrimal gland tissue of 150 day old male istar rats by use of a microrespirometer technique (Manhold, Bolden, and Ka Excrotory ducts were, then ligated., and the animals killed at 1, 2, 3, .4 days, 1 month, and 4 months poat-ligation. The duct-ligated glands were removed, placed in Tyrode's solution, and divided into two parts. Half was, fixed for microscopic study. Half was placod, in microrespirometers for Q_{O_2} determination. Results were: 1 day -(11 glands) 0.36+ 0.59; 2 days - (9 glands) 0.34+ 0.22; 3 days - (8 glands) 0.70+ 0.08; 7 days - (12 glands) 0.64+ 0,53; 14 days -(11 glanda) 0,50+ 0.14; 1 month -(10 glands) 0.29+ 0.25; 4 months - (10 glands) 0.50+ 0.10. Those results corresponded with the microscopic picture.. Tentatively, therefore, it may be concluded that duct-ligation caused an initial drop in the respiration of glandular tissue followed on the third day by an increase. A constant Q_{O_2} value, which is lower than the initial value, is reached when the gland histologically shows more scar tissue and less parenchym

Presented at the 39th General Meeting of the International Association for Dental Research,. Boston, Massachusetts,, March 1961.

COMPARATIVE STUDY OF SUBMAXILLARY AND LACRIMAL GLANDS OF THE RAT. Theodore E. Bolden and John. H. Manhold, Jr. Striking similarities in composition and response to disease processes have been demonstrated for salivary and acrimal glands. This tudy determined the composition and dry weight of acrimal glands and compared some of the data with ata obtained in a similar fashion for submaxillary glands. Lacrimal glands from seven groups of untated male Albino rats were used. (15, 30, 60 90, 150, 300 and 500 days.) quantitative estimate was made of the relative areas in sections of left ands which are occupiod by acinar and by intralobular duct tissue. An ocular grid divided into 66 squares was used A total of 1200 squares were counted. The number of squares in which half or more of the area was occupied by acini or ducts was counted in 3 pecimen from each age group. Dry weight determinations were made on the right gland of 27 rats from the last six ages. The relative proportion of acinar tissue to intralobular duct tissue remained fairly constant at all ages. The normal gland consisted of about 8% acinar tissue, and 3% intralobular duct tissue. Lacrimal glands consisted of approximately 24% more acinar tissue and 2 less intralobular duct tissue than submaxillary glands from rats of the same age. An average dry weight of 8 mlg. (30 days) was increased to 50 mig. (60 days) fell, and fluctuated round 25 mlg, at all other ages. The ratio of gland weight to body weight was 0,75 and .189 respectfully, for lacrimal and submaxillary glands.

Presented at the 39th General Meeting of the International Association for Dental Research, Boston, Massachusetts., March 1961.

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MICRORESPIROMETER TECHNIC FOR STUDY OF HUMAN GINGIVAL TISSUE: PRELIMINARY REPORT.

J. H. Manhold, Jr., T. E. Bolden, and S. Katz,
Seton Hall College of Medicine and Dentistry,
Jersey City.

Glickman, Turesky, and Hill (1949) employed the Warburg manometric technic to study human gingival metabolism. Because accurate Warburg determinations require relatively large quantities of tissue (40-50 mg. wet weight) and such quantities of human tissue usually are not readily available, Glickman, Turesky, and Manhold (1950) employed dogs to study oxygen requirements of healing gingival tissue. Exact relationships between human and dog gingiva Q_{O_2} values were not found. Further investigations did not follow. Kirk (1950) presented a quantitative ultramicroanalysis method for measuring the metabolism of tissue cultures. This technic, giving accuracy on 10 mg. or less (wet weight) of tissue, appeared ideal for human gingival study and was used in the present investigation. Small sections of gingival tissue, removed from clinic patients of Seton Hall College of Medicine and Dentistry, were placed in Tyrode's solution and divided into two approximately equal parts. Half was fixed for microscopic study. Half was placed in microrespirometers for Q_{O_2} determinations. Thirty-six samples of microscopically determined normal and essentially normal gingival tissue yielded a mean Q_{O_2} of 1.39 ± 0.46, a value comparable to earlier results. Microrespirometer investigation of human gingival tissue appears feasible, therefore, and presents the advantage of using small tissue quantities.

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J. D. Res., July-August 1960, Vol. 39, #4, pg. 746-7, #265.

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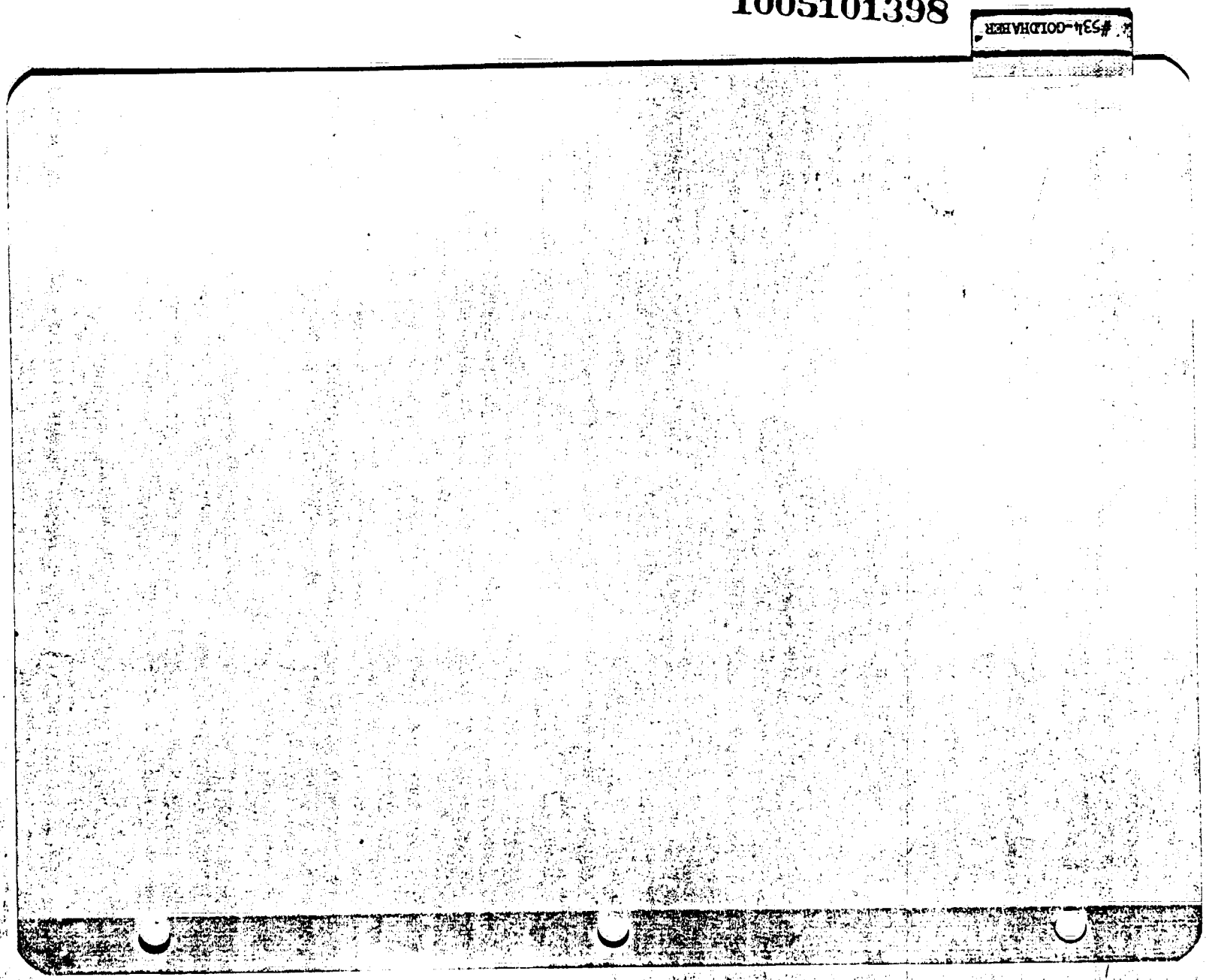

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A. CONFIDENTIAL February 14, 1966

MEMORANDUM

TO: Committee comprising Dr. Reimann, Chm., Dr. Andervont and Dr. Little.

FROM: Robert C. Hockett

SUBJECT: New grant application from Paul Goldhaber, D.D.S. and Herbert I. Bader, D.D.S. - No. 534.

We enclose herewith a new grant application from Drs. Paul Goldhaber and Herbert I. Bader of the Harvard School of Dental Medicine.

Dr. Goldhaber, one of the participants of the Oral Conference in October 1965, has submitted a proposal which entertains a very interesting investigation. Some preliminary work has been carried out which has indicated feasibility to be positive.

The question posed is simply stated: Does tobacco smoke condensate have antigenic properties (it appears to have such properties) and can well planned animal and human studies indicate the site in which either the antigen or antibody, locate and possibly have a biological effect?

Dr. Goldhaber and his associates are well qualified to undertake these studies. The unique approach to human studies and possible usefulness to future studies are most attractive.

If the grant is approved, the applicants will be briefed by the staff on the techniques of tobacco smoke production and the exposure of animals thereto, in the light of the Council's accumulated experience. Hence undue attention need not be given to the method mentioned on page (8) and elsewhere in the application.

R. C. H.

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(ORAL) CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

#534

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE

COMMITTEE:
Dr. Reimann, Chm.
Dr. Andervont
Dr. Little

638 THIRD AVENUE
NEW YORK, N. Y. 10017

Application For Research Grant

Date: February 3, 1966

1. Name of Investigator:

Paul Goldhaber*, D.D.S. and Herbert I. Bader**, D.D.S.

2. Title:

* Associate Professor of Periodontology
** Research Fellow in Periodontology

3. Institution & Address:

Harvard School of Dental Medicine
188 Longwood Avenue, Boston, Massachusetts

4. Project or Subject:

See detailed sheets

The Production of Antibodies to Tobacco Smoke Condensate or Smoke in Animals and Humans

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

See detailed sheets

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Budget Plan See detailed budget sheet

a. Salaries
b. Expendable Supplies
c. Other Expenses
d. Permanent Equipment
e. Overhead (15% of a, b, c)

Total

7. Anticipated Duration of Work: 5 years

8. Facilities and Staff Available:

a) microscopic equipment with ultraviolet light source.
b) photographic equipment calibrated for fluorescent microscopy.
c) working immunological assay systems.
d) animal quarters.
e) all the facilities of the Dept. of Oral Histopathology & Periodontology at Harvard School of Dental Medicine.

Consultation and advice, whenever indicated by the personnel of the Harvard Medical Community.

9. Additional Requirements:

none presently

10. Additional Information (Including relation of work to other projects and other sources of support):

Immunologic studies utilizing the fluorescent antibody technique as first developed by H. Coons [14], have been conducted in this laboratory for the past 3 years. These studies include both enzyme localization in tissues through the use of antibodies produced in rabbits against the enzymes in question and cytological localization of bacteria in human gingival tissues [16]. We feel there is direct applicability of the techniques developed in these studies to the problems posed in the present outline.

Currently, Dr. Bader's salary is derived from a U.S.P.H.S. Training Grant. His Fellowship will terminate June 30, 1966. The research supplies for the above mentioned research, equipment and technical assistance used are supported in part by the same training grant and a U.S.P.H.S. grant No. DE 01298 entitled "Further Studies of Mechanisms of Periodontal Disease" which is held by Dr. Paul Goldhaber.

Signature
Director of Project

Business Office of Institution Henry C. Meadow, Exec. Secr.
2 Committee on Research and Development

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4. Project

The Production of Antibodies to Tobacco Smoke Condensate or Smoke in Animals and Humans

On the basis of clinical observation, morbidity and mortality tables of smokers and non-smokers and other selected groups, tobacco smoking has been associated with various pathological changes in the human organism. Further experimental data is necessary to clarify this association. One neglected aspect of the problem concerns the antigenicity of tobacco smoke and the pathological changes that may take place in the host.

It may be noted that where one can find reference to studies of the immunology of tobacco it is primarily tobacco extracts and not the combusted products (smoke) that are used as the antigenic agents.

Pipes[1] in 1945 first suggested that any demonstrable immunologic response to proteins extracted from tobacco leaves may be quite apart from hypersensitivity to tobacco smoke. Rosen in 1953[2] and 1954[3] pointed out the differences between tobacco leaf extracts and absorption of tobacco smoke by inhalation. It is difficult to imagine tobacco protein remaining stable and unaltered following combustion and thus acting as an antigenic stimulus in its natural form in the smoker. Some attempts at elucidating the immunological effects of tobacco smoke were made by Armen & Cohen in 1958[4] by studying electrocardiographic changes in rabbits sensitized to tobacco extracts and challenged by smoke. While normal and sensitized animals did react differently, the ECG changes did not appear to be immunologically related. While others[5,6] have commented on the antigenicity of tobacco smoke particularly as an aerosol there is a definite lack of information in the literature clarifying its properties as a challenging or shocking antigen in animals.

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As mentioned earlier the majority of reports in the literature are based on clinical studies and most of these relate to cutaneous hypersensitivity as elicited by tobacco extracts. Within the past few years an increasing amount of interest has been shown in the role of tobacco allergy in thromboangiitis obliterans (TAO) and coronary artery disease[7]. There is ample evidence from the above-mentioned studies and others that immediate wheal and flare[8] and delayed type reactions[9] can repeatedly be demonstrated in tobacco sensitized individuals using, either tobacco leaf extracts or tobacco smoke condensate. While the above mentioned classical demonstrations of hypersensitivity can be duplicated in healthy individuals, patients with coronary artery disease and TAO have been shown to have a higher incidence of positive reactions to tobacco extracts[10,11], implicating in some fashion the possibility of tobacco allergy as being a factor in these cases. It is interesting to speculate on what role the immune system may play in the pathogenisis of the obstructive arteritis so typical of TAO (also known as Buerger's Disease)

Emphasis in the consideration of tobacco allergy as an accepted clinical entity and in the experimental work on the immunology of tobacco extracts[12] to this point in the discussion have all been based for the most part solely on the immunogenicity of the aterial (tobacco) without adequate investigation of other factors influencing host response. It is axiomatic that the ultimate immunologic response as judged by clinical or experimental criteria is to a great extent dependent upon the immunologic responsiveness of the host organism and the extent to which the response is mediated by existing disease, the nature of the antigen (e.g. - pharmacologic effects of nicotine) and any other parameters affecting the defense mechanism.

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In a review of the immunology of tobacco[13] there is evidence both for and against untoward influences on antibody formation by smoke and/or nicotine, the latter being

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non-antigenic although there may be some obscure haptenic activity. The pharmacologic effect of nicotine is a phenomenon quite apart from its antigenic properties. Tobacco smoke condensate if not antigenic in itself should be investigated for haptenic properties, particularly since it has been mentioned as having some properties as a shocking agent in eliciting anaphylaxis.

In summation, it appears the immunological aspect of smoking has been one of the most neglected phases of study in the relationship of tobacco utilization by the human organism. If we are to draw any valid conclusions or arrive at workable clinical concepts along this line these must be based upon fundamental immunological studies so as to provide a basis for expansion of our understanding of the nature of the tobacco smoke - host relationship.

If we accept the possibility that tobacco smoking may indeed be involved in the etiology of various diseases we still must explain why some smokers do not develop the disease in question whereas others do. It is conceivable that the difference may be in the immune response of the particular host to tobacco smoke. That is, the nature of the immune response may be the determining factor. Pathological changes may be the resultant of immune injury or may occur due to lack of immune protection. If the immune response is the key factor in precipitating the disease it may be possible to prevent or treat the disease in question by methods aimed at eliminating the responsible antigenic factor in tobacco smoke or by altering the host reaction. Furthermore, if our human studies indicate a correlation between the development of antibodies to certain components of tobacco smoke and the disease process, it may be possible to screen out those smokers who would fall into the category of "susceptible" individuals on the basis of their immunologic profile. Since non-smokers are known to develop all the diseases that have been associated with tobacco

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smoking we may postulate that the mechanism in those cases is similar although the antigen may be different.

With reference, therefore, to the paucity of experimental data on the immunogenicity of tobacco smoke, our long term goals are aimed at clarification of the immunological properties of tobacco smoke condensate in the human organism and to determine whether there is any correlation with various disease processes which seem to be associated with tobacco utilization. Specifically we intend to carry out antigenicity investigations in animals followed by cytological studies of antigen and antibody localization. At the same time we will study the nature of the smoke-host association in human subjects with emphasis on tissue changes as related to immune injury.

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Detailed Plans of Procedure

Experiments

1. Animal

Exp. 1.A Production of antibody in rabbits to tobacco smoke condensate.

Materials and Methods:

Ten female albino New Zealand rabbits about one year of age weighing between 2.5 3 kilos are divided into the following groups:

) 6 animals to receive antigen and adjuvant

b) 2 animals to receive salline and adjuvant

c) 2 animals to receive saline

ll animals are bled prior to immunization to insure non-reacti vity between their serum globulins andl the antigen (tobacco smoke condensate or TSC). The immunization schedule consists of TSC in 95% alcohol solution with a nitrogen content estimated by Lowry tech iq e of 4 mg/cc administered over a series of 10 injections, to give a total of 40 mg. of nitrogen. The initial administration of 4 mg. of antigen nitrogen is combined with an volume of Freunds complete adjuvant and given with 1/2 dose into toe pad! and 1/2 intraperitoneallly. One week folllbwing the intial dose the animals are given 4 mg. of antigen nitrogen I.V, for 3 successive days, rested 4 days and then repeated twice for a total of 10 doses. One week following the tenth administration of antigen the animals are bled from the marginal ear vein and the serum portion of the blood separated. The presence of circulating antibody in the serum will be determined by gel diffusion and complement fixation studies.

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Exp. 1.B Production of antibody in guinea pigs to tobacco smoke condensate.

Materials and Methods:

Ten large (450 grams) guinea pigs are used in the following manner:

a) 6 animals are to receive antigen and adjuvant

b) 2 animals receive adjuvant and saline

c) 2 animals receive saline only

The choice of guinea pigs is based on the fact that they are well suited to studies in hypersensitivity particularly when dealing with a material whose immunogenicity is an unknown factor. While the animal is not a good producer of precipitating antibody it is excellent for screening purposes since a number of very sensitive tests for antibody formation can be performed in the same type of animal in which the hyperimmune state is being attempted. Such easily demonstrable antigen-antibody reactions as passive cutaneous anaphylaxis (PCA) are good indicators of immunogenicity. In addition the animals are small, easily handled and relatively inexpensive. Tests for production of antibody will include complement fixation and passive cutaneous anaphylaxis (PCA).

Exp. 2 Cytological localization of site of antigen (TSC) in tissues by fluorescent antibody technique.

Materials and Methods:

Five albino female New Zealand rabbits, 2.5-3K to be subjected to smoke by forced inhalation in a specially constructed smoke chamber[4], consisting simply of a wooden box in which the test animal is subjected to smoke from a cigar forced into the area by a stream of compressed air. There are suitable openings in the box to prevent suffocation of the animal.

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Animals thus exposed are then sacrificed and specific tissues are quick frozen in isopentane in liquid nitrogen. The tissues to be selected are:

1) Nasal mucosa
2) Respiratory tract including bronchi and alveoli
3) Lymphatics, particularly hilar lymph nodes
4) Liver
5) Spleen
6) Oral mucosa, including gingivae
7) Heart

Immunofluorescent study of sites of antigen localization are conducted by the indirect method of Coons[14].

All antiserum is adsorbed before use with dessicated tissue powder for non-specific or non-reactive material.

Tissue samples are sectioned on a cryostat at 2-4 microns and air dried on a slide. Fixation in 95% ethanol at 37°C for 15 minutes is followed by washing in pH 7.2 phosphate buffered saline.

Test slides are then covered with the adsorbed antiserum and incubated in a moist chamber for 1/2 hour, washed and dried.

Goat anti-rabbit globulin conjugated with fluorescein isothiocyanate is then layered over the specimen and incubated in similarfashion, washed, dried and coverslipped with 10% buffered glycerol as mounting medium.

Slides are examined under ultra-violet illumination provided by an Osram HBO-200 mercury vapor bulb with appropriate 1° and 2° filtration.

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Positive test for presence of antigen in the tissues is bright apple green fluorescence at site of union of antibody with the antigen.

Controls consist of:

1) Antiserum reacted with antigen (TSC) prior to use on slide to absorb all specific antibody.

2) Use of normal serum on tissue should result in negative test.

Exp. 3. Immunofluorescent localization of antibody to tobacco smoke condensate.

Materials and Methods:

Two female albino New Zealand rabbits are sensitized to tobacco smoke condensate as described in experiment #1.

Animals are sacrificed and tissues named in experiment #2 are obtained and frozen.

Tissues are sectioned at 2-4 microns on a cryostate and air dried on slides.

(TSC) Tobacco smoke condensate is conjugated by the method of Riggs et al[15].

The conjugated (TSC - fluorescein isothiocyanate) antigen is then layered over the tissue sections as in the direct fluorescent antibody technique of Coons[14].

General procedure is as outlined in experiment number 2.

Union of labeled TSC with specific antibody localized in the tissues is visualized as a bright apple green fluorescence.

Controls will consist of inhibition of antibodies by layering sections with unlabeled antigen prior to use of conjugate.

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Exp. 4. Sensitization of animals to tobacco smoke by inhalation.

Materials and Methods:

Five albino female New Zealand rabbits weighing 2.5-3 kilos are used as experimental animals.

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Each animal to be placed in smoke chamber daily for 15 minutes and subjected to smoke as described in earlier experiment.

At end of one week and once weekly for a period of time up to one year (if necessary) blood is to be drawn from marginal ear veins and serum tested for presence of anti-tobacco smoke antibody by complement fixation and gel diffusion.

Exp. 5. Investigation of haptenic properties of tobacco smoke condensate.

As mentioned in preceeding discussion if immunologic response is not elicited by the use of TSC alone it may be possible to conjugate the condensate with a protein, and then in the immunized animal evoke a response by challenging with the TSC.

Therefore a regimen of injections similar to that outlined in experiment #1 would be carried out with a TSC - protein combination using guinea pigs as the test animals. Haptenic activity could be demonstrated in the sensitized animal by evoking a passive cutaneous anaphylactic reaction with TSC.

II. Humans

Exp. 1. Demonstration of tobacco-smoke antibodies in serum of human subjects. Subjects to be screened for presence of circulating antibody by means of complement fixation and gel diffusion techniques. Both smokers and non-smokers to be included in screening. A minimum of 100 subjects to be tested, at least 1/2 being considered "heavy" smokers by generally accepted criteria.

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Exp. 2. Immunofluorescent localization of TSC antigen in oral tissues of smokers. Experiment to be followed as outlined in animal experiments (utilizing indirect test).

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Exp. 3. Immunofluorescent Ibcalization of antibody to tobacco smoke in sensitized human subjects.

All smokers found' to have circulating antibody (in serum) to be further examined for presence of antibody in tissues with the direct fluorescent antibody test using TSC onjugated with fluorescein isothiocyanate.

ll tissues in experiments 2' and 3 are to be oral tissues obtained by biopsy or at surgical phase of periodontal therapy, frozen and prepared as described previously.

Exp. 4. Patch testing of selected groups for cutaneous sensitivity to tobacco smoke.

Selected groups of smokers and non-smokers (as mentioned in Human experiment # 1) be exposed to smoke as ai challenging stimulus by means of a cutaneous patch test. Observations to be recorded as either positive or negative hypersensitivity response.

All experiments are listed numerically and divided into two major groups e.g. animal nd human. This is not meant to imply order of procedure or relative importance. It should be noted that attempts to immunize animals against tobacco smoke condensate can and should be performed concurrently with screening of human subjects for anti-tobacco e antibody. In addition, cytological localization of antibody if any) is not ependent upon use of a prepared antiserum. As noted in experimental design an ntigen source is the only requirement for this type of study.

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BUDGET

Professional Personnel

Paul Goldhaber, D.D.S'., Associate Professor of Periodontology
Herbert I. Bader, D.D.S., Research Fellow in Periodontology 1 000
Soc. Sec. & Ret. Allow. 16% ,44 40

Non-Professional Personnel

Technical Assistance			
ecretarial Assistance			
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Consumable Supplies

Animals and animal care	00
hemical, reagents & glassware	600
Histological supplies	750
Photographic supplies	250
Smoke chamber, tobacco smoke condensate & cigarettes	200
Disposable syringes, needles, etc.	00 2,900

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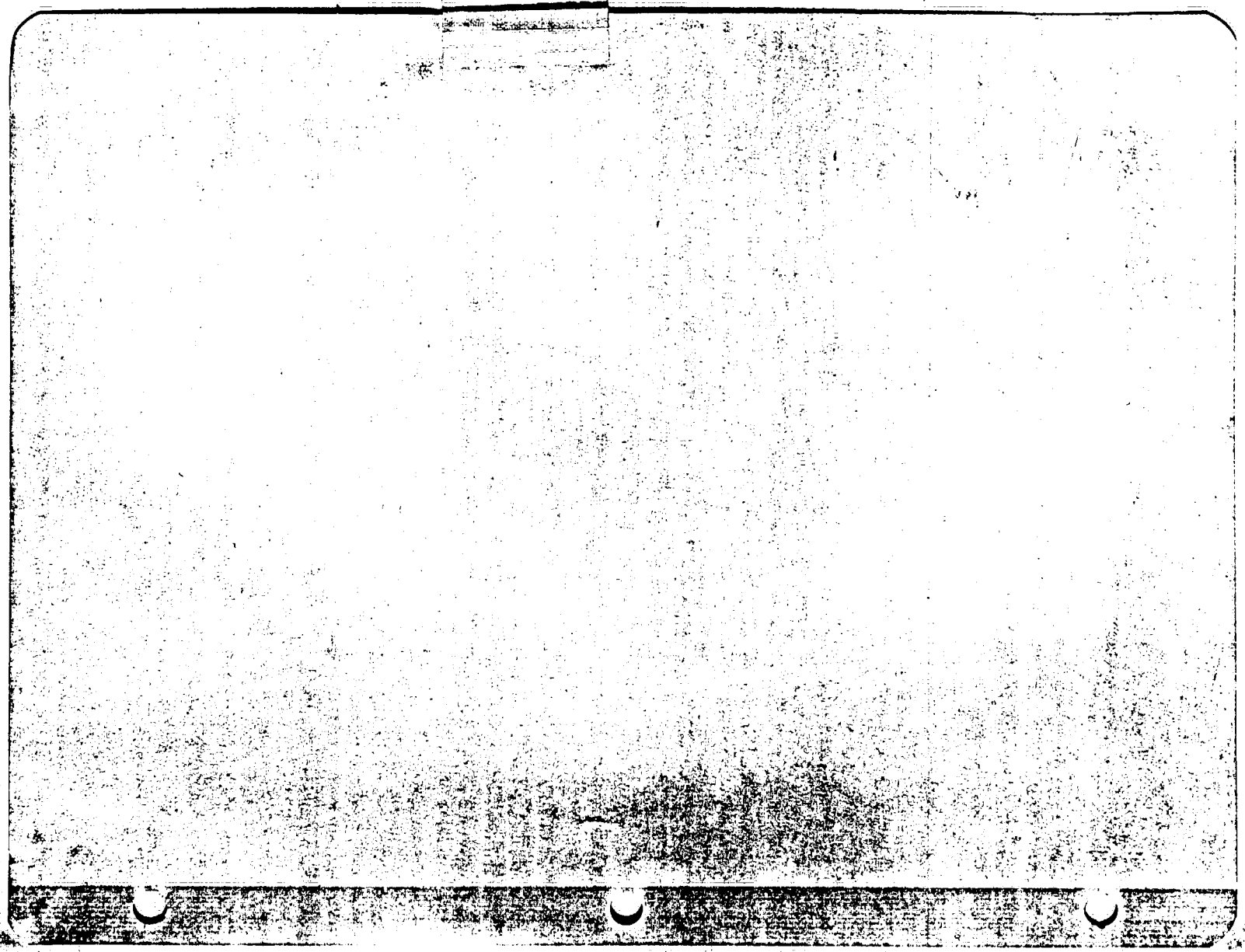

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THE COUNCIL FOR TOBACCO RESEARCH - U. S. A.

CONFIDENTIAL

February 17, 1966

MEMORANDUM

TO: The committee comprising Dr. Little, Chm., Dr. Andervont and Dr. Reimann.

FROM: Robert C. Hockett

SUBJECT: Interim grant application from Jerome F. Thomas, Ph.D. - No. 535.

We enclose herewith a request from Dr. Jerome F. Thomas of the University of California (Berkeley) , for an interim grant. The attached letter to Dr. Brady explains the situation and the reasons why a short-term grant is needed.

The subject is one relevant to our interests in that we too have been seeking a short-term screening technique for measuring "relative carcinogenicity" of various compounds and mixtures in a semi-quantitative manner. We have explored a number of possible methods and have found most of them unpromising. The one most similar in character to Dr. Thomas's was use of the so-called, photo-dynamic response of Paramecia to polynuclear hydrocarbons. The late Dr. Constantine Stephano attempted to develop this into a rapid and sensitive test on the basis of observations reported by Doniac. Dr. Robert W. Hull, then of the Department of Biological Sciences of Northwestern University, was given a grant (#305) beginning on April 1, 1961, for further refinement, standardization and evaluation of the test. Concurrently Dr. S. S. Epstein of Children's Hospital Medical Center in Boston undertook similar work under other auspices. Dr. Hull made substantial progress over a period of two years. He then transferred to Florida State University in Tallahassee and the second renewal grant was made to that institution as of September 1, 1963. No further reports have been received from Hull since his move.

A part of our original plan was to compare three different bioassay systems with use of the same hydrocarbons, all highly purified, carefully preserved and from the same source. The parallel bioassays were to have been by (1) Dr. B. A. Rubin's method (See grant #181) and (2) an accelerated skin test of Dr. R. K. Boutwell at Wisconsin, with purified hydrocarbons supplied by Dr. Ralph Becker of the University of Houston (#258).

The logistics of the integrated plan broke down mainly because the supply of highly purified hydrocarbons from Becker was inadequate for all three bioassays and Dr. Rubin had first call on the supply. Dr. Boutwell never carried out his part of the plan Rubin and Hull completed their studies independently. (Hull purified his own compounds).

In order to judge the status of our own further interests in this field, we need an up to date report of the state of Hull's work. Dr. Little telephoned him while I was on the way to the west coast. Hull promised to mail us a comprehensive report "next week", but it has not yet been received.

We have also endeavored for some time to interest our grantees in the field of tissue culture to investigate and describe the changes induced by carcinogenic hydrocarbons in cultured cells and endeavor to develop criteria for evaluating the transformation of normal cells to tumor cells. This, we thought, might develop into still another bioassay with some special merits. Dr. Pace (#516) has now finally started such work in the current year. I was not aware of Leo Sachs' papers when I last talked to Pace but will make sure that he is familiar with them.

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THE COUNCIL FOR TOBACCO RESEARCH - U. S. A. February 17, 1966

MEMORANDUM - Cont'd

In California I talked both to Dr. DeOme and Dr. Thomas. We wanted particularly to learn from the former, who is chairman of the Cancer Research Coordinating Committee of the University, whether Thomas is likely to receive favorable consideration of his project in May. DeOme indicated to me that he is prepared to recommend that his committee renew the grant to Thomas in May provided that he then has a concrete plan to compare his bioassay method with other more conventional ones or with such a method as that of Sachs. (Though he says that some other investigator was unable to confirm Sachs). Of course he does not know for certain whether the rest of the committee will go along with his recommendation.

Dr. Thomas told me that he is much interested in comparing his bacterial method with that of Sachs' and that Dr. Chen is qualified to do so and has actually begun work. The critical problem seems to be Chen's salary from January 1, 1966 until a new university grant is activated, if it is. Thomas would be quite willing to assay fractions of tobacco smoke condensate by his method as a quid pro quo if we provide interim support.

To summarize, Dr. Little and I would hesitate to recommend long-term support to this project unless or until we have fully taken stock of the progress achieved by Hull and by Pace. On the other hand we would look with some favor on a small grant to keep Thomas's staff intact while this evaluation is going on and pending the decision of the university committee as well as to get their comparative observations on some cigarette smoke condensates that have been assayed by our grantees using other methods.

The following papers submitted in support of the application are being sent to the committee only:

1. Manuscript entitled: Photobacterium phosphoreum as an indicator organism for carcinogenic activity. Chi-Sin Chen, and J. F. Thomas.
2. In vitro transformation of normal cells to tumor cells by carcinogenic hydrocarbons. Y. Berwald and Leo Sachs. J. Natl's. Cancer Institute 35, 641 (1965).
3. Inhibition of interferon production in hamster cells transformed in vitro with carcinogenic hydrocarbons. Z. Rotem, Y. Berwald and Leo Sachs. Virology 24, 483 (1964).
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R. C. H.

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in the same hydrocarbon class as benz(a)pyrene, a carcinogen - all are atmospheric pollutants.) Further, the atmospheric pollutants are collected only in such minute quantities that assaying for carcinogenic activity by conventional techniques is impossible.

The original thought was to find a single micro organism that could be added to the list of conventional test animals, such as mice, rabbits, dogs, etc. Coupled with a simple test, with the index for carcinogenic activity being based on induced morphological or biochemical variations compared to the control. The advantages of using micro organisms are (1) simplicity; (2) rapidity; (3) only minute quantities of test material are required; (4) relatively inexpensive; (5) close environmental control; (6) a large number of "test animals" with respect to generations and populations easily maintained and readily available for test purposes.

Bacillus megaterium was the original organism selected using relatively few compounds of known composition for the developmental work. An attempt was made to force the organism to indicate according to established carcinogenic activity of the compounds, by modifying the environment, i.e. those compounds classed as carcinogenic were to cause the organism to behave in a manner quite different from organisms exposed to non-carcinogens or the control. (A paper has been published on this work.) It soon became apparent that a single organism would not satisfy if simplicity and rapidity were to be considered as essential to the developmental objectives.

It does appear feasible to develop a number of simple tests using a variety of organisms. A large number of test compounds have been gathered (list included) and the list is being gradually increased. It is hoped that a sufficient number of individual tests may be developed so that any compound giving, for example, three positive tests out of a possible ten total tests will be at least statistically indicative of carcinogenic activity.

Work is currently being done using (1) Photobacteria phosphoreum (ATCC 11040), (a paper has been prepared for publication covering this work to date); (2) Pasteurella tularensis (ATCC 6223), Lysogenic, Dept. of Bacteriology, University of California, Berkeley; (3) Bacillus megaterium; (4) an "unknown rod" isolate from sewage (A Pseudomonas-like organism found to grow in the presence of 5 percent acetone, allowing for increased solubility of test hydrocarbons). Emphasis is being placed on finding general and specific enhanced or retarded enzyme activities as indices of carcinogenic activity although many morphological and biochemical variations occur simultaneously which are also being given cursory examination for possible future exploratory work. At the suggestion of Dr. DeOme part of the screening should include a simple tissue culture aspect to serve as a bridge relating the findings of these

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atypical tests to the more conventional assay techniques. (Tentative arrangements have been made to use some facilities at the U.C. Medical Center, S.F. for the tissue culture work.)

Monies are requested to enable the work to continue along the bread exploratory paths now being followed.

VIII. Although the major objective has been to develop a screening technique for assaying carcinogenic activity of minute quantities of material, the studies to date involve work on single cell organisms in a completely controlled environment which looks very promising as leading to an insight to chemical carcinogenesis in higher animals.

IX. Facilities and equipment of the major air pollution project are available for use by this small auxilliary project. Help is required only for salaries and expendable supplies as indicated in the budget. A proposal is being prepared for submission to the Public Health Service to carry this research as an entity in itself.

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SECTION II -- PRIVILEGED COMMUNICATION

Continuation page

1. RESEARCH PLAN

A. Introduction and Specific Aims

(1) Description

Mammalian animals are conventionally used to assay carcinogenic activity. These assay techniques are not sensitive enough for use with suspect atmospheric pollutants because only small quantities of test material are available. In recent years there has been an indication that microorganisms sensitive to minute quantities might be used for an alternate assay system. Probably not a single organism but a select group must be used for screening because of different metabolic patterns of the different organisms.

The advantages of a microbial assay system include: 1) simplicity; 2) rapidity; 3) only minute quantities of test material are required; 4) relatively inexpensive; 5) close environmental control; 6) large number of "test animals" easily maintained and readily available for test purposes.

Several microbiological assay techniques have already been developed incorporating growth response, enzyme activity, photodynamic action and induced morphological and cytochemical changes. Each individual test appears promising but each also gives anomolous results with certain compounds.

The general objective of the proposed work is to develop new techniques and improve existing techniques so that each carcinogen will give two or more positive tests using a screening program involving a number of test organisms.

Preliminary work on bacterial enzyme activity has also indicated several parallels to more conventional cancer research, which might eventually give some insight into the mode of carcinogenesis.

(2) Rationale

The basic assumption is that carcinogenic compounds will affect microorganisms differently than structurally similar noncarcinogenic compounds. A variety of isomeric compounds must be included in the test program to be sure the indicating effect is due to carcinogenic activity and not size, molecular weight or other variables of little consequence.

The rationale behind the basic assumption may be summed up briefly in five points (i through v). These are presented in outline form and in expanded detail under the pertinent literature references in Section 2.C.

Possible indicating effects of carcinogens on bacterial cells:

1) Indirect effects: (i) In photodynamic action, the carcinogens serve as sensitizing agents. Recognized at cellular level--death of the cells. (ii) The carcinogens change cellular surface to permit or stimulate variants to grow. Recognized at cellular level--formation of heterogenic population or predominant variants.

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2) Direct effects: (iii) The carcinogens change gene(s) in a cell by interacting with DNA. Recognized at cellular level--formation of mutants and heterogenic population. Recognized at subcellular level--change in metabolic patterns and enzyme deletion. (iv) The carcinogens interact with enzymes and non-enzyme proteins. Recognized at subcellular level--change in metabolic pattern and enzyme deletion. (v) The carcinogens participate in induced enzyme formation (adaptive enzymes). Recognized at subcellular level--inducing synthesis of TPNH-dependent enzymes.

(3) Specific Aims

The research to be undertaken will be directed toward the establishment of microbialindicators of carcinogenic arene type hydrocarbons using induced atypical physiological and biochemical responses with special emphasis on enzymatic activities both at intact and subcellular levels. An outline of the study plan follows with details being given under methods of procedure (Section 1. B.).

1) To evaluate the growth rate of microorganisms as indicator for carcinogenic effect with special references to stimulatory effect on facultative, anaerobic organisms and inhibitory affect on aerobic organisms.

2) To investigate the biochemical aspects of carcinogenic effect upon the microbial system by comparing the following characteristics in call treated with carcinogens and with non-carcinogens.

) Intact cellular level

) Oxygen uptake using Warburg nmanometric technique

) Rate in utilitation of glucose--quantitative wet chemical analysis with respect to time

i) Rate in uptake of inorganic phosphate--quantitative wet chemical analysis with respect to time

Subcellular level

i) Activities of some enzymes involving carbohydrate metabolisms --classical methods will be followed

Activities of enzymes containing active sulfhydryl group

iii) Activities of TPNH-dependient enzymes

iv) Isolation and characterization of metabolites using chromatography

3) To select the enzymes highly associated with tumor cells in animals and to evaluate their activities in microbial system treated with carcinogens.

) To evaluate and determine photodynamic activities in microbial system.

5) To examine the affect of the hydrocarbons on morphological and cytochemical aspects of the bacterial cells.

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B. Methods of Procedure (References refer to Pertinent Literature References listed under section 2. C.; see p. 14)

Approximately 50 compounds will be used in the development and evaluation techniques. Primarily arene type hydrocarbons associated with polluted atmospheres will be used; a list is included in Table I (these compounds are currently available, being used in conjunction with other research currently in progress in this laboratory related to air pollution). A variety of sizes, shape, number of condensed rings and molecular weights are included among the compounds including a number of carcinogenic and non-carcinogenic isomers.

The species of bacteria to be studies include 1) Photobacterium phosphoreum (ATCC 11040), 2) Pasteurella tularensis (ATCC 6223), 3) Bacillus megaterium (lysogenic, Department of Bacteriology, University of California), 4) an "unknown rod" isolate (Pseudomonas-like organism). This organism, isolated from sewage, was found to grow in the presence of 5 percent acetone media. (Incorporation of the acetone is to increase the solubility of testing hydrocarbons.) Identification of this latter organism will be made.

I) Growth Aspects. Liquid media will be used for studying the effects of incorporated hydrocarbons on growth. The growth will be determined turbidimetrically using wavelengths of 600 mμ for medium containing peptone and 400 mμ for colorless medium. Hydrocarbons will be added as an acetone solution. With exception of the "unknown rod" all the acetone will be removed from the medium in vacuum oven at 50°C. Individual compound will be tested first and later various combinations of carcinogens and non-carcinogens will be made to see any effect on growth by interaction between or among the hydrocarbons. All the results will be expressed in per cent growth based on the control sample. Triplicate tubes for each treatment will be made.

Since 100 per cent differentiation of carcinogens from non-carcinogens may seem impossible, therefore two or more assay organisms may have to be teamed up for a systematic screening of the carcinogens.

II) Biochemical Aspects. (A) Enzymatic studies: Assays will include both intact cells and cell-free systems.

a) Intact cells: All the assaying bacterial cells preincubated with or without the testing hydrocarbon will be harvested and the test will be made in the presence of the hydrocarbon. Specific activities of each enzyme assay will be compared between cells treated with carcinogens and with non-carcinogens. Specific activities will be expressed as: activity per unit number of viable cells or activity per unit dry weight. Viable counts will be made by diluting cell suspension to a proper concentration and the dilute suspensions will then be plated out in triplicate with suitable agar and incubated at proper temperature for certain period before counting. The dry weight of a cell suspension will be made by drying aliquots of the cell suspension in 105°C oven for overnight.

b) Cell free preparation: Bacterial cells preincubated with or without the testing hydrocarbons will be harvested and the cell free enzyme will be prepared by sonic treatment, grinding with alumina oxide or glass. Testing hydrocarbons will

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SECTION II -- PRIVILEGED COMMUNICATION AP 00469-01

BLE I. LIST OF AVAILABLE TESTING HYDROCARBONS

1. Anthracene	8. Dibenzo(ghi, pqr)perylene (coronene)
2-methylanthracene	9. 20-methylcholanthrene
9-methylanthracene	0. pheranthrene
9, 10-dimethylanthracene	1. chrysen. (benzo(a)phenanthrene)
Benz(a)antbracene	2. Benzo(a)chrysene (piecene)
3-metbylbenz(a)anthracene	3. Dibenzo(b, k)chrysene
4-methylbenz(a)anthracere	4. Dibenzo(def, mno)chrysene
3-methylbenz(a)anthracens	S. Benzo(1)phenanthrene
4-methylbenz(alanthracene	6. Benzo(c)phenanthrene
0. 5-methylbenz(a)anthracene	7. Dibenzo(a, i)phenanthrene
11. 6-metbylbenz(a)anthracene	8. Pyrene(benzo(def)phenanthrene)
7-methylbenz(a)anthracene	9. Bcnzo(e)pyrene
3. 8-rnethylbenz(a)anthracene	0. Benzo(a)pyrene
4. 9-methylbenz(a)anthracene	1. 5-methylbenzo(a)pyrene
5. 10-methylbenz(a)anthracene	2. Dibenzo(a, e)pyrene
6. 9,10-dimethylbenz(a)anthracene	3. Dibenzo(e, 1)pyrene
7. 7, 12-dimethylbenz(a)anthracene	4. Dibenzo(a, h)pyrene
18. 2-phenylanthracene	5. 5-rnethyldibenzo(a, h)pyrene
9. 9, 10-diphenyhnthracene	6. 5,10-dimethyldibenzo(a,h)pyrene
0. Dibenz(a, c)anthracene	7. Fluoranthene
1. Dibenz(a,h)anthracene	8. Benz(e)acephenanthrylene
22. Dibenz(a,j)anthracene	9. Benzo(k)fluoranthene
3, Benz(b)anthracene (Naphthacene)	0. 11 H-benzda)fluorene
4. Benzo(b)naphthacene (pentacene)	1. 11 H-benzo(b)fluorene
25. Benzo(a)pentacenc	2. Z,4,7-trinitro9-fluorenane
26. Dibenz(de,kl)anthracene (perylene)	3. 9-fluorenane
7. Benzo(ghi)perylene	4. 5,7-dimethylbenz(a)acridine

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then be mixed with the enzyme for short period so the interaction between the hydrocarbons and the enzyme can take place. With this system, permeability barrier is eliminated. Substrates and other cofactor will be added just before the assay. The specific activity will be expressed as activity per unit dry weight or activity per unit weight of protein. The protein determination will be made using Lowry's[1] Folin-Ciocalten method. Egg albumin will be used for preparing standard curves.

Assay procedures for various enzymes studies on intact and cell-free systems will be made by spectrophotometric and manometric methods. A tentative list of enzymes to be studied includes: 1) Enzymes of carbohydrate metabolisms:[2] glucose-6-phosphatase, fructose-1-6-diphosphatase, phosphoglucomutase, phosphohexose isomerase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate-dehydrogenase (these enzymes are highly associated with animal tumor). 2) Enzymes containing active -SH groups:[3] triose-phosphate dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase. 3) TPNH-dependent enzymes:[3] aldehyde dehydrogenase, benzaldehyde dehydrogenase, 5-dehydroshikimic reductase, glucose-6-phosphate dehydrogenase glutathione reductase, isocitric dehydrogenase, malic enzyme, nitrite reductase. 4) Amino acid enzymes associated with animal tumor:[4] treonine dehydrase, histidase, α-kete glutaric dehydrogenase. 5) Others: Catalase.[5]

(B) Respiration studies: Previous studies of Ph. phosphoreum has shown that carcinogens suppress the respiration of the organism compared to non-carcinogens. The respiration rate was measured by dimming time method. Further tests employing addational test compounds and using manometric technique[6] for measuring oxygen consumption will be made. Cells preincubated with the carcinogens will be used for the assay. Total uptake of oxygen in unit time and in unit dry weight will be expressed as specific activity for comparison.

(C) Uptake of glucose and inorganic phosphate: Glucose is utilized as an energy source, and inorganic phosphate is provided for phosphorylation. Test of the uptake of these two components in the presence of testing hydrocarbons in liquid medium will probably furnish information concerning energy problems. A certain period will be allowed for the uptake of the glucose and the inorganic phosphate; the remaining glucose and inorganic phosphate will be determined so that the net uptake of both can be estimated. The Lowry-Lopez method[7] will be used for the phosphate determination and the "glucostat" method of Worthington Biochemicals, Freehold, for glucose.

(D) Analysis of metabolites: The microorganism will be grown on agar plus testing hydrocarbons in petri dish or Roux cultures bottle. The harvested cell will be washed and solubilized with NaOH or hypochlorite. The metabolized hydrocarbons are released so that they can be extrated with organic solvent. Chromatographic methods will be extensively employed for separation and characterization of the metabolites. Gas chromatography will be used primarily for the hydrocarbon metabolites and the more volatile intermediate meterbolites normally associated with cellular growth. Paper column and thin layer chromatography will be employed in areas that gas chromatography is not applicable.

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III) Photodynamic Study. Bacterial cell suspension in the presence of testing hydrocarbon will be exposed to ultraviolet light and the total viable count will be estimated before and after exposure to the light. Per cent survival will be calculated on the basis of viable cell before the exposure. Comparison on per cent survival will be made for different testing hydrocarbons at different length of exposure.

IN) Morphological and Cytochemical Studies. Morphological and cytochemical studies will be made by staining technique for differentiation of shape and cytochemical changes. Qualitative changes of the cells caused by the carcinogens will be evaluated.

. Significance of Research

1) Several relatively simple microbial techniques for assaying air pollutants for carcinogenic activity have already been developed. Although each technique fails for a number of test compounds there is an excellent possibility that a number of simple systematic tests might be combined into a broad screening assay techmque. Thus the microbial techniques appear promising as alternates to the conventional animal tests.

) An evaluation of degree of carcanogenic activity among isomrrs, with klose environmental control, can be readily made using the microbial systems.

3) The biochemical approach of the present proposal involves fundamental tudies on enzymatic activities of bacterial cells treated with carcinogenic hydrocarbons. The induced atypical bacterial cells may be comparable to cancer cells. Therefore the studies may lead to an insight of the chemical carcinogenesis in higher animals.

4) There is potential possibility of modifying or using present microbial systems for screening antitumor agents.

D. Facalities Available

Existing and fully equipped and staffed laboratories are available within the Sanitary Engineering Researcb Laboratory, University of California, Berkeley. Library and consultants of the highest quality are readily available.

Specific equipment currently available and in use includes incubators, ovens, autoclaves, balance, microscope, refrigerated centrifuge, sonic oscfflators, spectrophotometers, Warburg's apparatus in addition to minor equipment. A low temperature incubator, vacuum oven and certain chromatographic equipment are currently being used on a short loan basis.

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2. SUPPORTING DATA

A. Previous Work

This laboratory has been engaged in the field of air pollution for a period of over ten years resulting in numerous publications concerned with the identification and resolution of atmospheric pollutants. Interest in assaying many of the resolved, unidentified compounds for carcinogenic activity was aroused in the early work. For example the clear resolution of an atmospheric sample by means of chromatography led to the ultimate identification of about 20 per cent of the compounds with many of these corresponding to known carcinogens. Many of the chromatogram peaks adjacent to the carcinogens could not and still have not been identified. Should an extended effort be made toward a more complete analysis on the basis of potential carcinogenecity? It became apparent that more sensitive assay techniques were needed for carcinogenic activity.

Previous work in this laboratory on developing more sensitive assay techniques involved the use of plant seedlings as the assay organisms introducing the test compounds hydroponically. The results were presented at a Symposium on Air Pollution sponsored by the American Chemical Society in 1963 but never published. The results were promising in that all compounds behaved as expected but the experiment was considered not satisfactory because over 60 days was required for the test.

Parallel work with bacteria appeared more promising and a publication eventually resulted.[8,9] Copies are being sent under separate cover. An aerobic organism Bacillus megaterium, was used in this work. Considerable effort was made at normalization and standardization of the technique but again the results were not completely satisfactory. A slight decrease in the dissolved oxygen had a comparable effect on the growth rate compared to the inhibitory effect of the carcinogens. A discussion of this effect is included under pertinent literature references (Section 2. C.).

Effort was shifted toward another organism, Phtobactrium phosphorium, a facultative anaerobe. The amount of dissolved oxygen was not limiting toward the growth. (A publication covering this research is currently being prepared and a rough draft is being sent under separate cover.) On this work the respiration rate was found to be indicative.

Another aspect considered under current work is the solubility of the test material in water. The low solubility of aromatic hydrocarbons in water might minimize the carcinogenic effect. To evaluate enhanced solubility a chemically defined medium plus glucose and 5 per cent acetone was inoculated with sewage. Three organisms were isolated of which one is currently being investigated. Relatively large amounts of carcinogen may be introduced in the acetone.

Biochemical, enzymatic and growth studies comparable to the herein described techniques are currently under investigation.

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B. Personal Publications

" matic Hydrocarbons Produced From Simple Aliphatic Fuels, " Mukai, M., Thomas, J. F. , Tebbens, B. D., Anal. Chemistry, 37, 398 (1965).

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" ltidimeusional Chromatography of Arenes Produced During Combustion, " Mukai, Mi, Tebbens, B. D., Thomas, J. F., Anal. Chem., 36, 1126 (1964).

Fluorescent Spectra of Aromatic Hydrocarbons Found in Polluted Atmospheres, " Thomas, J. F. , Tebbena, B. D. , Sanborn. E. N. , Cripps, J. M., Int. J. of Air Pollution, 2, 2I0 (1960).

. Pertinent Literature References

The present investigation is primarily interested in the effect of chemical carcinogens on microbial cells. A summary of pertinent literature has been organized into S categories and is discussed below.

Point 1: Photodynamic action of carcinogens on unicellular paramecium has been extensively studied in recent years. 10 Although the exact nature of the mechanism has not been uncovered, the system has received considerable attention serving as a practical method for detecting carcinogens. The possibility of using bacteria as photodynamic assay organisms has been recognized using UV light on Saccharomyces carlbergensis in the presence of benzo(a)pyrene. 11 Recently Beebe and Pirsch~ reported that Pasteurella species were killed at a rate proportional to the light intensity. The role of carcinogens in photodynamic action of Paramecium has been proposed as a sensitizing agent; tests of Pasteurella in similar conditions may also provide a useful indicator for the carcinogenic activity.

Point 2: According to "colbnal' selection theory, "13 a carcinogen is considered to be capable of alternating the cellular environment so that the preexisting cellular variants are stimulated or permitted to grow. There ts no direct biochemical pathway between the carcinogens and the hereditary information. A review of the literature has indicated a general pattern of microbial growth response toward chemical carcinogens. A summary is presented in Table 2. The growth of facultative anaerobic organisms is either stimulated or not affected by carcinogenic hydrocarbons. In contrast aerobic organisms are always inhibited by the carcinogens. Research in this laboratory also supports this conclusion.8,9

Point 3: The "instructional theory"13 considers a carcinogen as a mutagen which participates directly in cytochemical alternation of the cells and eventually leads to irreversible change in hereditary informational' content of the affected cell. Evidence ofinteraction between carcinogen and nucleic acid has been demonstrated in animals. 14, 15 Nucleic acid in bacterial cells are also affected in the polynuclear carcinogenic hydrocarbon. The ratio of RNA/DNA in Escherichia coli treated with the carcinogen was shown to be lower than cells treated with non-carcinogens, 16 since DNA constitutes genes, the effect of the carcinogen on nucleic acid may lead

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TABLE 2. EFFECT OF CARCINOGENS ON MICROORGANISMS BASED ON THE GROWTH RATE AND ON REACTION TO OXYGEN

Growth[1]	Tested Organisms	Relation to Oxygen	Carcinogens Tested	Dosage ug/medium	Incubation Period	Degree of Effect	References
Inhibition	Bacillus megaterium	aerobic	benz(a)pyrene	0.2 ug/ml	20 hr.	---	Applied Microbiology 10:217-222, 1962
	Lactobacillus casei	microaerophic	p-amino methyl amino azobenzene	1-3 ug/ml	---	---	Cancer Research 7:730, 1947
	Chaetomium sp. (mushroom)	aerobic	dibonz(a,h) anthracene	20 ug/ml	2 days	---	Growth 9:177-187, 1945
	Fusarium lini	aerobic	dibenz(a,h) anthracene	saturated solution of u.v. exposed compound 1 ml/100 ml	5 days	---	Proc. Soc. Exp. Biology and Medicine 38: 370-372, 1938
	Vibrio choleae	aerobic	na-salts of dibenz(a,h) anthracene and 20-methyl cholanthrene	32 ug/ml	20 hrs.	---	J. Infectious Disease 89: 59-75, 1951
	Streptococcus pyogenes	facultative anaerobic					
	Streptococcus salivarius						
	Staphylococcus aureus						
	Corynebacterium diphtheria						
Stimulation	Escherichia communion	facultative[3]	dibenz(a,h) anthracene and 3-methyl cholanthrene	300 ug/ml	9 hr.	50%	Science 86: 176, 1937
	Escherichia communion	facultative	dibenz(a,h) anthracene	---	9 hr.	---	J. Bacteriol. 38:13-23, 1939
	Escherichia coli	facultative	dibenz(a,h) anthracene and 3-methyl cholanthrene	water saturated solution	---	19-36%	J. Bacteriol. 44:394, 1942
	Staphylococcus aureus	facultative					

SECTION II -- PRIVILEGED COMMUNICATION (Continuation page)

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able 2 (cont'd)

rowth	ested Or anisms	Relation to xygen	arcinogens ested	oiige /medium	ncubation eriod	egree f Effect	eferences
tim- lation	accharomyces llipsoideus	acultative	-methyl holanthiene	2 ug/ml	---	--	nn. Missouri ot.Garden
							4:583-589,
							937
	scherichia coli	acultative					. Nat. Cancer
o ffect							nst. 10:11-27,
							949
	accharomyces	acultative					nn.Missouri
	llipsoidous						ot.Garden
							28:1-30, 1941

rowth compared to the control treatment

oth carcinogens tested were in the forms of Na-Salts which could behave differently from th. riginal arcinogens

Facultative anaerobic organisms

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to mutation of the cell. The mutaganic nature of the carcinogen has been tested by susceptibility of the E. coli to bacterial phage. Non-carcinogens do not show any mutagenic properties.[17] Prolonged exposure of various bacterial cells to polynuclear carcinogenic hydrocarbons has induced changes in morphology and growth rate.[18,19]

Both the "colonal selection" and "instructional" theories eventually deal with heterogenic populations predominanted with variants or mutants which in the pathological sense are equivalent to tumor cells in the higher animal.

Point 4: Although it is commonly accepted that molecular structure of a protein is a literal translation of DNA molecules, yet the presence of a given DNA molecule in a cell does not always produce the corresponding enzyme(s) because the gene expression is controlled by feedback systems, i.e., "negative feedback" and "repression." From a biochemical viewpoint, uncontrollable proliferation of cancer cells signifies a possible upset in metabolic regulation of the cellular activities. A general practice of pathological studies is to compare enzymatic activities in diseased cells and in normal cells. Such examination is made to reveal the failure of enzyme forming systems as well as the inactivation of certain enzymes associated with the disease. In the animal system cancer cells can be induced by aromatic hydrocarbons which were demonstrated to interact with the cellular protein.[20] Histological studies in rat tissue have revealed that the carcinogens may affect the Sulfhydryl group of the protein in tissues.[21] Since sulfhydryl groups constitute the active groups in some enzymes, question is naturally raised whether carcinogenic hydrocarbon can specifically react to inactivate sulfhydryl group of the enzyme or can cause alternative metabolic pathways by changing the enzyme structure resulting in "enzyme delation."[22] Information concerning inactivation of enzymes containing active sulfhydryl groups, such as lactic dehydrogenase in muscle[23] and alternation of metabolic pathways in animal tumor cells[24] has been reported. Corresponding studies on microorganism has been very limited. Some preliminary biochemical studies on the effect of carcinogenic aromatic hydrocarbons on Bacillus megaterium have been reported.[9] The results indicate that the hydrocarbons affect the dehydrogenase as shown by methylene blue reduction. The organism also accumulates lactic acid in the cells treated with carcinogens. It is hoped that the interaction between carcinogenic hydrocarbons and the cellular components in the microbial system can be shown and the resulting effect may reveal qualitative and quantitative aberration of an enzyme activity (or activities). The enzymatic aberrations caused by the carcinogens in the microbial system may eventually serve as a model for the studies in animal carcinogenesis.

Point 5: Studies in liver microsome in recent years have indicated that the activity of TPNH-dependent enzymes can be markedly influenced by carcinogenic hydrocarbon such as benz(a)pyrene.[25] The carcinogen stimulates the synthesize of the enzymes. Similar studies on microbial system have not yet been done. Likewise, some of the highly active enzymes associated with cancer cells in animals have not yet been evaluated in the microbial system treated with carcinogens. This is a field of great promise in cancer research, because a relationship between tumors of higher organisms and equivalent "tumors" in the microorganism may be linked through this lane of approach. Investigation in this direction may yield data of comparative physiological significance to the higher form.

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SECTION B - PRIVILEGED COMMUNICATION

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3. BIOGRAPHICAL SKETCHES

(Give the following information for EACH key staff member, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	DEMOGRAPHIC (Mo. Day Yr.)
Jerome F. Thomas	Professor of Sanitary Engineering	REDACTED
PLACE OF BIRTH (City, State, Country)	PRESENT NATIONALITY (If non-U.S. citizen, indicate visa status)	SEX
Chicago, Illinois	USA	☒ Male ☐ Female

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED
DePaul University, Chicago, Illinois	B.S. (Chem)	1943
University of California, Berkeley	Ph. D. (Chem)	1950

HONORS

MAJOR RESEARCH INTEREST

Research in area of water pollution
Research in area of air pollution

RELATIONSHIP TO PROPOSED PROJECT

Principal Investigator

RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Starting with present position, list ALL experience relevant to project)

Actively engaged in teaching and research in the field of air and water pollution for the last 15 years. Between 40 and 50 research papers have been published in scientific journals. Faculty Investigator for numerous master's and doctorate candidates in the field of sanitary engineering (chemical aspects of air and water).

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SECTION II -- PRIVILEGED COMMUNICATION

Continuation page AP 00469-01

Name: Chi-Sin Chen Title: Assistant Research Microbiologist

REDACTED

Place of Birth: Li-Kang, Taiwan, China

Visa Status: H-1 visa (3 year), Distinguished Visitor Sex: Male

Education: National Taiwan University, Taipei, Taiwan, China, B.S., 1957
Agricultural and Mechanical University of Texas, College Station, Texas, M.S., 1962
Oregon State University, Corvallis, Oregon, Ph.D., 1965

Major Research Interest: Studies on microbial physiology with special interest in enzymatic reactions. Research work on microbial biochemistry.

Relationship to Proposed Project: Direct and perform major bench aspects of proposed work

Research and/or Professional Experience:

Full time research for past 8 months on the project related to the present proposed work. Work on Master's and Doctorate thesis.

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BERKELEY: CANCER RESEARCH COORDINATING COMMITTEE
OFFICE OF THE EXECUTIVE SECRETARY
230 EARL WARREN HALL

January 3, 1963

J.R. Thomas
Department of Sanitary Engineering
Berkeley

Dear Doctor Thomas

The Cancer Research Committee has reviewed your application, and I regret to inform you that your request has not been approved.

It is the sense of the Committee that the interpretive problems underlying exposure of bacteria to hydrocarbons are too great to approve a new grant application in this subject without full discussion by the Committee. It is therefore suggested that your request be resubmitted when the annual notices are sent out in February for consideration of applications by the Committee at its regular spring meeting.

Yours sincerely,

DE COMS
Executive Secretary
Cancer Research Coordinating Committee

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SANITARY ENGINEERING
RESEARCH LABORATORY
RICHMOND FIELD STATION

1301 SOUTH 46TH STREET
RICHMOND, CALIFORNIA 94804

11 January 1966

J. Morrison Brady, M. D.
Associate Scientific Director
The Council for Tobacco Research - U.S.A.
633 Third Avenue
New York, New York 10017

Dear Dr. Brady:

Enclosed is a copy of the research proposal I discussed with you over the telephone when you were in Seattle. It is concerned with the development of a bioassay screening technique for carcinogenic activity of compounds available only in minute quantities, such as atmospheric pollutants. The proposal was submitted to the Cancer Research Coordinating Committee, University of California (USPHS), of which Dr. K. B. DeOme is the Executive Secretary. Also enclosed is a copy of the letter postponing any further consideration for financial assistance until the regular spring meeting which occurs in early May.

Dr. Chen and I were in to see Dr. DeOme yesterday to clarify the position of the proposal. In summary he stated that the proposed work dealt primarily with the use of microorganisms as the "test animals" in the analysis. He felt that emphasis should be shifted to the establishment of a bridge between conventional assay techniques, i.e., the direct application of relatively gross amounts of test materials into mice and the proposed microbiological technique. In fact he stated that monies would most likely have been provided if we had indicated more interest in work on the bridge between the conventional and microbiological techniques.

We discussed this bridge work in some detail and concluded that emphasis in our work for the next few months should be shifted in this direction. Dr. DeOme suggested using the work of Dr. Leo Sachs as the model: Dr. Sachs has reported that the carcinogenic hydrocarbons, benzo(a)pyrene and methyl cholanthrene can directly induce in vitro, using secondary tissue cultures of hampster embryo origin, the transformation of normal cells to tumor cells. Non-carcinogenic hydrocarbons

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such as chrysene and pyrene do not cause the transformation. We would apply the technique to a large number of hydrocarbons (See Table I, page 6 under Research Plan) and statistically evaluate the results. Dr. DeOme has been in personal contact with Dr. Sachs and is very enthusiastic about our checking the work with a large number of compounds. He has indicated that based on his discussions with Dr. Sachs, both a qualitative and quantitative analysis is possible. Copies of the work of Dr. Sachs, et al., are included for your perusal.

As I mentioned in our telephone conversation, the proposed research is an outgrowth of our air pollution project which is concerned with combustion at atmospheric pressure and the analysis of combustion products. We have separated from the atmosphere a large number of pollutants in minute quantities that fall in the same hydrocarbon class as benzo(a)pyrene but are still unidentified. The proposed project is without funds and I have managed to meet the salary of Dr. Chen on a monthly basis and have reached the end of my resources.

Dr. Chen received his Ph. D. in microbiology in June of 1965 and has taken this work on in a postdoctorate capacity. Of necessity I will have to close the project unless it is possible to receive some immediate financial help to carry the salary of Dr. Chen through June of 1966. I am firmly convinced that we will be able to obtain support monies shortly through more conventional channels as the project has shown considerable progress and merit to date. This is actually a cry for help before the project closes. I do not believe I could find another microbiologist interested enough to work as part of our research group to carry this phase of air pollution work along.

I have turned to you for help because of a parallel need in the tobacco industry for an assay technique applicable to the minute quantities of material that might be encountered in tobacco smoke.

Please feel free to call and discuss the problem in greater detail if you feel it warrants consideration on your part. I would also like to repeat my invitation to visit our campus and air pollution research facilities during your stay in San Francisco in May. Perhaps a luncheon at the Faculty Club can be arranged. Incidentally, I will be in New York to present a paper related to air pollution at the TAPPI (Paper & Pulp) meeting at the Hilton Hotel during the week of 21 February if you would care to discuss any phase of the proposed work.

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The amount of money requested is the first item under VI Budget ($6,100.00). Supply and expense money can be drawn from another source.

Very truly yours,

Jerome F. Thomas, Ph. D.
Professor of Sanitary Chemistry
University of California (Berkeley)
Th 5-6000, Ext. 3269

JFT:fkm

Enclosure

cc - Dr. K. B. DeOme

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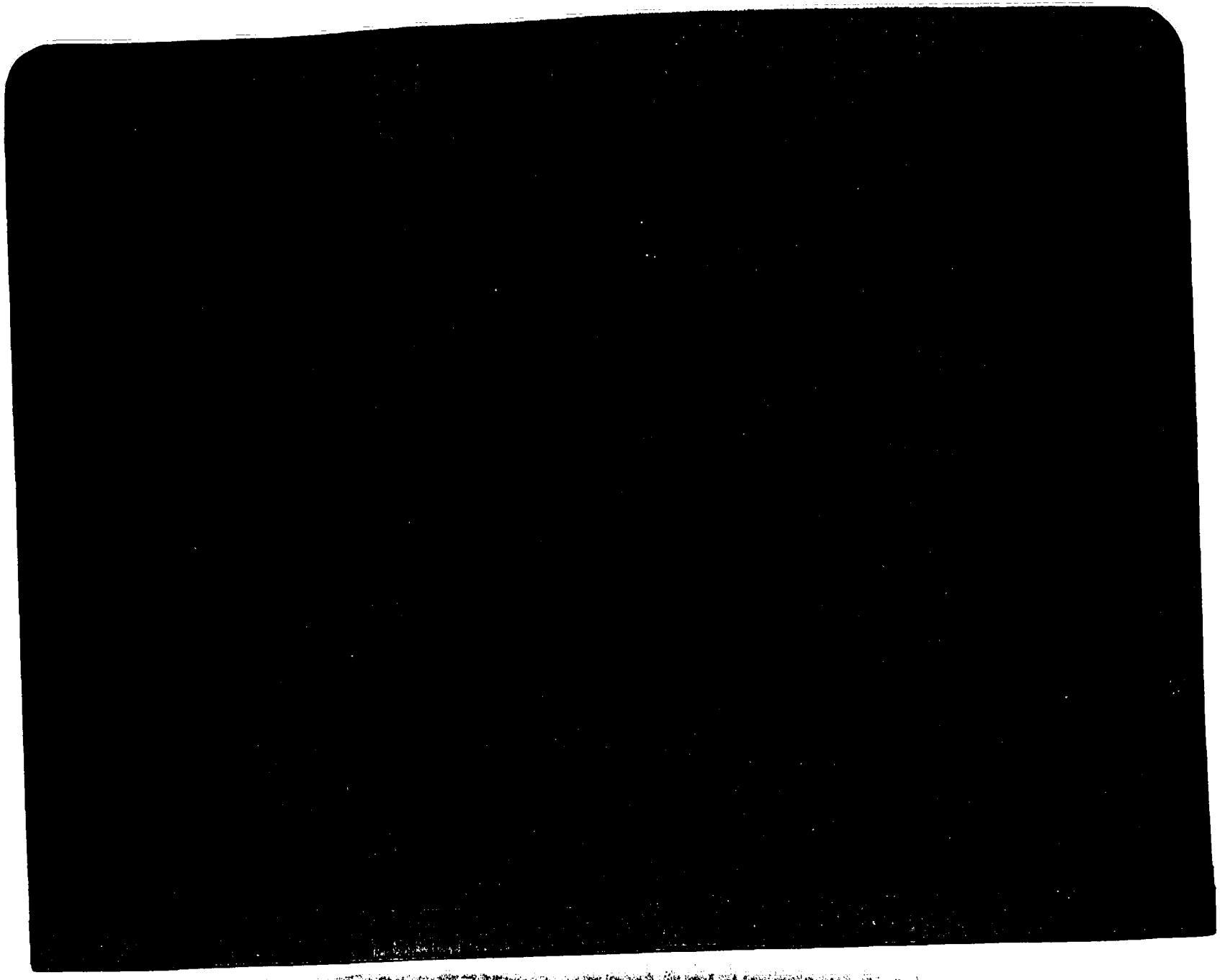

Source: https://www.industrydocuments.ucsf.edu/docs/nlwm0000